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THE INFLUENCE OF THE TREATMENT OF SYPHILITIC PREGNANT WOMEN UPON THE INCIDENCE OF CONGENITAL SYPHILIS

By J. Whitridge Williams

In a paper upon *The Value of the Wassermann Reaction in Obstetrics*, which appeared in October 1920, and which was based upon the study of 4547 women whose Wassermann reaction had been determined during the four years ending December 31st, 1919, I considered the significance of the Wassermann reaction during pregnancy. At that time it was shown that in our material syphilis constituted the most important single factor concerned in the causation of fœtal death, and was responsible for 34.4 per cent of all deaths occurring between the period of viability and the expiration of the first two weeks of the puerperium. It was, furthermore, shown that the results of treatment were highly satisfactory. When no treatment was instituted 48.5 per cent of the children manifested signs of syphilis, as contrasted with 39.2 per cent and 6.7 per cent, when the treatment was inefficient or efficient, respectively.

Intensive work along these lines has been continued, and has led to a great reduction in the incidence of fœtal syphilis in our service. Moreover, in the routine histological examination of the placenta syphilitic lesions are now relatively rarely encountered, instead of very frequently as in the past. In the two years following the completion of the series just referred to, that is, from January 1st, 1920 to December 31st, 1921, 96 of the women included in it have passed through 113 pregnancies in the service, and it has seemed to me that it might be interesting to follow their outcome, especially in regard to the effect of treatment; more particularly as no one has as yet studied a series of syphilitic women who have been under continuous observation for a number of years. Furthermore, the conclusions reached should be especially valuable, as it has been attempted to follow as far as possible the history of all the children concerned. In April and May

of the current year every child that could be traced was visited, so that we have notes on the condition of a large number of the children 4 to 28 months after their birth.

It might be added that in both series the Wassermann tests were made and the treatment instituted in the Syphilitic Department of this Hospital. In talking with Dr. Albert Keidel, concerning the results obtained, he suggested, and I agree with him, that in the former article the differentiation between efficient and inefficient treatment was based upon somewhat uncertain premises and possibly led to erroneous conclusions. In order to insure greater accuracy I have, at his suggestion, classified the treatment under one of the five following groups: A. The administration of at least six doses of diarsenol, followed by mercurials and iodides, and repetition of the treatment until the Wassermann became negative and remained so for at least one year after the cessation of treatment. B. Patients in whom the Wassermann remained persistently negative for six months after the last treatment. C. Patients in whom it did the same for three months after the last treatment. D. Patients in whom a negative Wassermann was attained only shortly before the birth of the child; while Group E includes all patients in whom the treatment was notoriously insufficient, or was not followed by the conversion of a positive into a negative Wassermann, no matter how long it was continued.

Upon analyzing the 113 pregnancies occurring in the 96 women during the two years under consideration, it was found that 9 had ended in abortion and 6 in premature labor. In the former the children were not viable, while in the latter they were either born dead or died during the puerperium, thus leaving 98 pregnancies for consideration. Such an incidence seems high, and at first glance one might be tempted to regard it as a result of syphilis, were it not for the fact that current clinical statistics teach that in normal women at least every fifth pregnancy ends in abortion, which is higher than the incidence here noted. Moreover, upon studying the abortions more critically, it was found that one was induced on account of severe chronic nephritis in the mother. Three occurred in the early months before the effects of syphilis could become manifest, while five occurred between the fifth and sixth month. In three of the latter, autopsy showed no signs of syphilis, and the placental and X-ray findings were likewise negative; while in the other two, autopsies were not obtained but the placentæ were normal on histological examination. In four of the six premature labors, the autopsy and placental findings were negative; while in the other two an autopsy was not obtained but the placental findings and maternal Wassermann were negative. Accordingly, while it cannot be categorically stated that syphilis played no part in the production of any of the fifteen abortions or premature labors, it nevertheless seems permissible to assume that it did not.

Of the 98 children remaining, four died from accidents of labor—one premature separation of the placenta, one prolapse of the umbilical cord and two craniotomies. In each instance, autopsy showed no sign of syphilis. On the other hand, four other children were born dead or died during the puerperium and showed signs of congenital syphilis, thus leaving 90 living children which were discharged from the clinic in apparently good condition.

During the months of April and May (1922) strenuous efforts were made to locate these children, and owing to the persistence of Dr. John G. Murray and our prenatal nurses all but nine of them were found, and were subjected to careful physical examination and a specimen of blood was withdrawn for a Wassermann. This must be regarded as a surprising achievement when the character of the clientele of the service is taken into consideration, and it is recalled that many of the patients had moved several times since leaving the clinic.

Of the 81 children which could be traced, 71 were found to be living and well, manifested no signs of syphilis, presented a negative Wassermann, and were apparently in excellent condition. In another instance, the child could not be traced after the end of the third month, but as it presented a positive Wassermann at that time, as well as at birth, it must be regarded as syphilitic. Of the remaining 9 children, five were living and apparently well, but their mothers would not allow blood to be withdrawn for a Wassermann; while four others had died—three from pneumonia at periods varying from 7 to 14 months after birth and one from gastroenteritis at the age of 11½ months. From these figures, it is apparent that of the 94 children who were born alive, 5 or 5.3 per cent showed demonstrable evidence of syphilis, while 76 were living and well, 9 had been lost track of, and four had died.

The results obtained are graphically shown in Table I which represents an analysis of the 113 pregnancies. In the first five lines in the second column the type of treatment employed is indicated, while in the last two the notation "no treatment" or "spontaneous cure" is made. In the former it may be stated that 13 pregnancies occurred in 12 women, and a word of explanation is necessary concerning the lack of treatment. Ordinarily, it was due to the fact that the patients had registered so late in pregnancy that labor occurred before treatment could be instituted, and sometimes even before the result of the Wassermann test became known; while in other instances the patients had neglected or refused treatment. The eleven pregnancies under the caption of spontaneous cure occurred in nine women, who were not treated for the reason that the Wassermann was already negative, and the history indicated that the disease had disappeared. In these women, after the birth of a varying number of syphilitic

children, the Wassermann had become negative without treatment and subsequently all children were normal. In one patient in the series, this had occurred as early as 1912, while in others the negative Wassermann dated from 1915, 1916, 1917 and 1918 respectively. Such spontaneous cures substantiate the observations of the older writers and indicate how cautious one must be in attempting to evaluate the results of treatment in a given case.

TABLE I.

Analysis of Treatment in 113 Pregnancies.

No. of Cases.	Type of Treatment.	Child L. & W. Wass. negative.	Child L. & W. No Wassermann.	Born alive. Wass. + later.	Born alive. Lost track of.	Congenital syphilis.	Died after discharge.	Born dead. No signs of syphilis.	Premature stillborn.	Abortions.
16	A	10	—	—	1	—	—	—	2	3
13	B	10	—	—	1	—	(74) 1	—	—	1
11	C	9	—	—	—	—	(78) 1	—	—	1
20	D	13	3			(64) 1	(64) 1	(17) 1	1	—
29	E	19			2	(50) (100) 2	(40) 1	(15) (109) 2	(88) 1	2
13	No treat.	2	2	1	2	(44) 1	—	(103) 1	2	2
11	Spont. cure	8	—	—	3	—	—	—	—	—
113	TOTAL	71	5	1	9	4	4	4	6	9

(The figures in parentheses refer to the serial numbers of the cases.)

The results obtained by the varying types of treatment are graphically shown in another way in Table II. Here it will be noted that the 49 women, who had received type A to D treatment, had gone through 257 pregnancies in the two series. Of these, 172 occurred before and 85 after the institution of treatment, and resulted in the birth of 40.7 and 89.4 per cent of live children, respectively. In this connection it should be noted that we have classified abortions as well as premature labors as dead children. Before the patients came into our hands we had no means of determining the cause of fœtal death; so, if similar allowances are made in each group, it seemed that gross figures would give a more correct idea of the results before and after treatment. It should also be noted that a number of these women were treated only in the previous series and received no treatment subsequent to 1920, others were treated in both series, while still others were treated only after January 1920. In either event the results are striking, and indicate that the incidence of dead children and abortions was six times less after than before treatment.

Moreover, comparison of the results recorded in Tables I and II indicates that type A to C treatment gave almost ideal results, while in Group D they were surprisingly and unexpectedly good. Furthermore, the results noted in Group E were in some respects even more startling, as they show that, from 29 pregnancies occurring in 18 women, 19 normal children were obtained, as demonstrated by examination four to twenty-eight months after birth, notwithstanding the fact that the treatment had been notoriously inefficient when judged by the usually accepted standards. Indeed, in several instances it was noted that the administration of only two or three doses of diarsenol had apparently sufficed to produce a negative Wassermann and to result in the birth of live children.

TABLE II.

Results of Treatment—96 Mothers.

Type of Treatment.	Number of Women.	Pregnancies before Treatment.	Deaths before Treatment.	Pregnancies after Treatment.	Deaths after Treatment.
A	12	47	26	20	3
B	11	39	27	15	0
C	8	28	20	14	2
D	18	58	19	36	4
	49	172	102	85	3
		59.3%		10.6%	
E	26	103	46	48	6
		44.6%		12.5%	
No treat.	12	66	29 + 1 syphilis alive		
		43.9%			
Spont. cure.	9				
	96				

What conclusions can be drawn from the data just adduced? It must at once be admitted that it is difficult to make any precise statement, except that our figures clearly indicate that almost ideal results follow anything like efficient treatment of syphilitic pregnant women, and that surprising results may sometimes follow what would ordinarily be regarded as altogether inefficient treatment in men or in non-pregnant women, which would seem to indicate that pregnant women are unusually amenable to anti-syphilitic treatment. On the other hand, too roseate a view of the possibilities of treatment should not be indulged in; for, while surprising results have been obtained in many patients, others were encountered who were extraordinarily refractory to treatment;—as for example, Case 23, to whom 30 doses of diarsenol were administered without bringing about a negative Wassermann. Notwithstanding this, the child showed no evidence of syphilis and when examined nine months after delivery presented a negative Wassermann.

Such favorable results as we have recorded appear to offer another example of the extent to which syphilis, as it occurs in women during pregnancy, differs from that occurring at other times, as well as in men, and indicates

that there must be something about the pregnant condition which mitigates the virulence of the disease and predisposes to spontaneous cure. Observations tending to support such a proposition were made by Keidel and Moore in studying their patients with neuro-syphilis, when it was noted that extension to the nervous system was less frequent and much more benign in women who had had children than in nulliparous women or in men. Furthermore, all who have had experience in dealing with syphilitic pregnant women, have come to believe that the disease is much more benign in them than in others, and tends in a certain measure to spontaneous cure. Moreover, experiences of this character are in line with the observations of Wade Brown that in experimental animals the pregnant female is very refractory to inoculation with syphilis, while non-pregnant controls are readily infected by the same virus, and tend to indicate that there is something about the pregnant female which seems to interfere with the extension of the spirochete.

At this time it appears futile to discuss the factors which may be concerned in such reactions, but it is apparent that a large field is opening up, which offers unusual opportunities for experimental study to those fitted for it. On the other hand, the practical lesson is that the results already attained afford abundant evidence that the syphilitic pregnant woman is unusually amenable to treatment, and this fact should encourage all who are interested in prenatal work to make every effort to recognize the existence of syphilis and to treat it during pregnancy, as by so doing a normal child may not only be obtained, but the mother can apparently be cured with far greater ease than at any other time.

In this connection a few words may be said concerning the diagnosis of syphilis in pregnant women. From my experience with my own assistants, as well as from what I gather elsewhere, it must be admitted that there exists a general tendency to base the diagnosis almost exclusively upon the presence of a positive Wassermann reaction and to neglect the clinical study of the patient. In my previous papers I pointed out that the presence of a persistently negative Wassermann on the part of the mother did not preclude the existence of syphilis and the possible birth of syphilitic children, and I have insisted upon the necessity for the greatest care in taking the history and searching for stigmata of the disease. Unfortunately, however, the results obtained in this respect are not very encouraging; as in the 96 women under consideration in this report only 12 presented a history of frank syphilitic infection, while in five others a suggestive history was obtained. Consequently, with the exception of these few cases, we were obliged to base the diagnosis either upon the existence of a positive Wassermann or upon the autopsy findings in the child.

Within the last two years our diagnostic facilities in the case of the newly-born have been greatly enriched by the work of Shipley and Pearson on the use of the X-ray, and we have had abundant opportunity to confirm its value. Naturally, if an autopsy can be obtained and is done by a competent man, the X-ray findings are merely confirmatory, but if an autopsy is not permitted, or the child is still alive, they offer a very important means of diagnosis, as in our experience the characteristic shadow of Wegner's bone disease at the junction between the diaphysis and epiphysis of the long bones must be regarded as pathognomonic.

In *conclusion*, I am aware that the present paper is based upon observations made upon a series of women value, but at the same time the results which we have obtained are highly suggestive and are interesting from two main points of view. First, because our study is based upon observations made upon a series of women with whose past history we have long been familiar, and secondly, because the results obtained are extraordinarily stimulating from the standpoint of treatment and of the great hope which it offers for the future.

THE USE OF LARGE REVERDIN GRAFTS IN THE HEALING OF CHRONIC OSTEOMYELITIS

By Mont R. Reid, Cincinnati, Ohio

(From the Surgical Department of the Johns Hopkins Hospital)

The many methods that have been introduced for treating bone cavities following operations for chronic osteomyelitis, attest to the difficulty of dealing with this surgical problem. In general they have been based upon the principle that obliteration of the bone cavity is desirable, and may be grouped under three headings:

(1). *Autoplastic operations with the use of soft tissues.* This idea perhaps originated with Neuber who, in 1886, described a method of treating bone cavities by turning into them flaps of skin and other soft tissue. This principle has been widely employed. (2). *Autoplastic operations with the use of bone.* This procedure is essentially the same as the preceding, except that the overhanging edges of bone are used together with the fascia and skin to fill the cavity. (3). *Obliteration by the use of various filling materials.* Schede introduced the method of healing by means of the moist blood-clot; Mosetig Moorhof used a paste; Senn employed decalcified bone chips, and various surgeons have used fresh detached pieces of fascia, bone, fat and muscle.

The purpose of this paper is to introduce another method of treating certain bone cavities, remaining after operations for chronic osteomyelitis, which the above procedures too often fail to cure. It consists essentially of epithelializing the walls of bone cavities by the use of large Reverdin or pinch grafts. J. P. Lord, in 1902, employed a Thiersch graft in a similar way, but so far as I know, the use of pinch grafts in the treatment of bone cavities has not hitherto been reported. The value of the two methods of grafting will be discussed after the report of two illustrative cases.

CASE 1.—Surgical No. 50382. A white man, aged 37, was admitted December 29, 1919. He complained of swelling, pain and lameness in the left leg.

The infection, beginning acutely in the upper end of the tibia, had been present for twenty years. During all this time, periods of quiescence of the infection with healing of the sinus were followed by periods of pain and swelling of the leg which were relieved by the discharge of pus and sequestra.

Operation, December 31, 1919. The cavity in the upper end of the tibia, measuring two inches in length and half an inch in diameter, was found filled with granulation tissue. This was removed with a curette. An attempt was then made to obliterate the cavity by cutting away its bony walls, but its position in the tuberosity of the tibia and the proximity of the knee-joint made this impossible. The operation terminated, therefore, in an attempt to secure healing by Schede's moist blood-clot method.

After three weeks, during which the healing of the wound seemed quite satisfactory, there developed signs of a mild infection. This subsequently necessitated the opening of the wound and the removal of the infected blood-clot. The cavity was then treated by the Carrel-Dakin method. When it became lined with healthy, relatively sterile granulation tissue it was epithelialized by the use of thick pinch-grafts removed from the thigh.

Since the patient's discharge from the hospital March 21, 1920, there has been no further trouble with his leg (*vide* Fig. 1).

CASE 2.—Surgical No. 49232. A white man, aged 55, was admitted August 6, 1919. He complained of "bone disease in the right leg."

The infection of the bone had begun when the patient was 9 years old, and for forty-six years his leg had many times discharged pus and fragments of bone. Periods of incapacity had varied from several months to a year. During the many years of his affliction the only surgical treatment of the disease had consisted in the incision of superficial abscesses.

FIG. 2 shows the great increase in the size of the right leg, the numerous scars of previously discharging sinuses, and, what has often been noted in long-standing cases of osteomyelitis, the increase in the length of the leg. In this instance the right tibia measured 3 cm. longer than the left.

Operation, August 8, 1919. Numerous small abscesses each containing a tiny sequestrum were present throughout the diseased portion of the tibia. The process was so extensive that the operation resulted in the production of a large boat-shaped cavity which extended from the tuberosities to the malleolus.

From August 8 until September 10 the wound was treated with Dakin's solution. The granulating surface was then covered with thick pinch-grafts which promptly grew and covered the surface of the cavity with epithelium.

February 17, 1920 (*vide*. Fig. 3). The cavity was well lined with skin which showed no tendency toward maceration. The photograph, unfortunately, does not show the limits of the upper end of the cavity which extended far into the tuberosities of the tibia.

Technique of skin grafting.—The bone cavity is treated with Dakin's solution until it becomes lined with clean firm granulations. Without the use of Dakin's or some other antiseptic solution the granulation tissue becomes œdematous or "sea-weedy," and forms an unhealthy base for the growth of grafts. Two hours after the last irrigation with Dakin's solution large thick pinch-grafts, half a centimeter in diameter are placed closely together upon the surface of the cavity. The grafted wound is then exposed to the air for from six to eight hours. This short drying period serves to fix the grafts firmly to the granulation tissue. The grafts are then covered and held in place with a single layer of gauze which is firmly secured to the normal skin so that the moistening and changing of saline compresses during the next two days will not displace the grafts. After two days the grafts have taken and the use of Dakin's solution instead of salt solution is begun. This is applied by laying wet compresses directly against the wound (the thin layer of protecting gauze having been removed) every two hours during the day and every four hours at night. After about five days the Dakin's solution is discontinued and the wound is dressed with rubber "protective" or old linen. Invariably the grafts grow quickly and cover the granulation tissue with epithelium in from ten days to two weeks. Should the granulations become high (usually from the œdema of infection) caustics should not be used; Dakin's solution reapplied for one or two days will reduce the swollen granulations to the level of the grafts, and will not injure the growth of new skin.

Indications for skin-grafting.—The cure of chronic osteomyelitis when the disease is confined to the shaft of a long bone is usually not difficult, for the surgeon can frequently remove bone enough to prevent the formation of a cavity or else to permit soft tissues to fall readily into a shallow cavity. But in the ends of long bones, particularly the upper and lower ends of the tibia and occasionally the lower end of the femur, the successful treatment of a bone cavity is made difficult by the presence of the joint which limits the amount of bone that may be chiseled away. In such locations the surgeon has always to face the problem of dealing with a deep cavity, and it is in such cases that we have for the past four years made use of thick pinch-grafts.

Again, when the shaft of the tibia is greatly enlarged, a thorough removal of all sequestra and foci of infection may often result in a cavity that cannot be obliterated by the removal of bone or by the infolding of the scant soft tissues that cover this bone. One occasionally encounters a similar condition in the humerus. Whenever, therefore, we have a large bone-cavity that is difficult to treat by other methods, the use of pinch-grafts makes it possible

to secure a rapid epithelialization of the wound.* (*vide* Figs. 2, 3.)

We have adopted the use of pinch-grafts for several reasons. The epithelial covering which results is thicker and more durable than that obtained by the use of Thiersch grafts. In deep cavities there is always a tendency toward the formation of moisture which will macerate delicate epithelium, and in the bone cavities of which we have been speaking, we have found that the thick pinch-grafts are tough enough to withstand this tendency to maceration, whereas Thiersch grafts often appear to melt away after taking.†

Another factor in favor of the use of pinch-grafts is the certainty of their taking. On a surface properly prepared with Dakin's solution practically every graft will take. Thiersch grafts, on the other hand, do not grow as constantly, for the impossibility of sterilizing completely a granulating surface will often result in the accumulation of pus which may lift these grafts from their base.

Changes in the size of the grafted bone cavity.—The fate of the epithelialized bone-cavity has been the subject of an interesting study. For several years we have been interested in watching the changes that take place after grafting deep wounds of the soft parts. Ample opportunity for this study has been afforded by the cases of carbuncle which it has been our practice to excise completely and graft within a few days. In this way, we have produced many epithelialized depressions of the soft tissues. Nature corrects to a remarkable degree this cosmetic blemish, for after several months the normal contour of the body is re-established to a surprising degree. This subject will be dealt with more fully in a paper on wound healing and wound contraction. It is mentioned here because of a similar process which takes place in epithelialized bone cavities. The patient upon whom Lord operated wrote after returning home that the cavity in his bone was much smaller. Ignorant of this observation, Dr. W. F. Rienhoff and I began studying the changes in the size and shape of the bone cavities in our cases. This has been done by making plaster casts of the cavities before grafting and at frequent intervals afterward. In Fig. 5 is shown a photograph of a series of casts from one patient. The leveling of the sides and the decrease in the depth of these cavities form a striking example of nature's attempt to obliterate a cosmetic defect. Whether or not epithelialized bone cavities may be completely obliterated has not yet been determined.

With such a method of treating chronic osteomyelitic bone cavities the time required for curing the most stubborn cases is usually from four to six weeks. This means a great economic saving. Considering the chronicity of this disease and the difficulty of curing it, the resultant deformity is of no moment to the patient. In a recent case in which the patient had suffered for thirty years and the ankle joint had become completely ankylosed, I grafted a large cavity running entirely through the ankle (*vide* Fig. 6).

Everyone is aware of the liability to recurrence of the infection after any of the other methods of treating chronic osteomyelitis. In the method I am proposing there should be but a relatively slight chance for recurrence; but that it may not recur must for the time being remain an undetermined point.

* Another application of the method has been useful in a few cases of extensive acute ostemyelitis of the tibia when, at the time of the primary operation, it has seemed necessary to split the periosteum throughout its length. In each instance the periosteum quickly retracted and the wound presented the appearance of extruding the shaft of the tibia. After from four to six weeks the removal of the sequestered tibia left a relatively flat involucrum covered with granulation tissue. These wounds were treated with Dakin's solution and then epithelialized with the aid of large Reverdin grafts. The results have been the substitution of flat bones for the normal tibiæ. In the few cases that have been followed these new-formed bones have tended to become round.

† Reverdin's original grafts were very small. He describes them as epidermic grafts. However, he points out that his grafts included the whole epidermis and very little of the dermis. By preparing a more nearly sterile granulating surface with Dakin's solution we can at the present time successfully transplant very large Reverdin grafts. In the center of these large grafts the entire thickness of the skin is included. Though differing from Reverdin's grafts in that they are larger and thicker, the principle remains the same and the present day pinch-grafts should still, I think, be called after Reverdin.

REFERENCES

Lord, J. P.: Jour. Am. Med. Assn., May 31, 1920, vol. 38, p. 1433.

Neuber, G.: Arch. f. klin. Chir., 1896, LI, 683.

Nichols, E. H.: Jour. Am. Med. Assn., Feb. 13, 1904, vol. 42, p. 438.

Senn: Amer. Jour. Med. Sci., 1899, N.S. XCVIII, p. 219.

Mosetig-Morrhof (von): Deutsche Ztsch. f. Chir., 1904, LXXI, 419.

Reverdin: Bull. d. e. Soc. impériale de chir. de Paris, Dec. 1869, pp. 493-511.

Arch. gén. de méd., 1872, pp. 276, 555, 703.

Gaz. méd. de Paris, 1871, 3 s., 26, p. 554.

Bull. gén. de thérap., etc., Paris, 1872, LXXXII, 71-74.

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FIG. 1.—Case 1. Surgical No. 50382.
Johns Hopkins Hospital.

On the thigh are the scars that resulted from cutting the pinch grafts. In the bone cavity the grafts are still visible. Photograph taken fourteen months after the patient's discharge from the Hospital.

FIG. 2.—Case 2. Surgical No. 49232.
Johns Hopkins Hospital.

Duration of the disease 46 years. Note the increase in size and length of the right tibia.

FIG. 3.—Case 2.

Several months after epithelialization of the bone cavity. The extent of the cavity into the ends of the tibia is not shown in the photograph. The grafts can be seen.

FIG. 4.—Surgical No. G2812. Cincinnati General Hospital.

Bone cavity is shown completely epithelialized two weeks after being grafted with large pinch grafts.

FIG. 5.—Surgical No. 53038. Johns Hopkins Hospital.

Plaster molds of an epithelialized bone cavity; 1 day before, 5 days after, 18 days after, 42 days after and 10 months after skin grafting. The decrease in the size of the cavity is plainly shown in the molds.

FIG. 6.—Surgical No. 56249 Johns Hopkins Hospital.

The joint was ankylosed by a chronic osteomyelitis of thirty years duration. The arrow shows an epithelialized cavity extending through the destroyed joint.

FIG. 7.—Surgical No. 55878. Johns Hopkins Hospital.

Bone cavity three weeks after grafting it with large pinch grafts.

THE MORPHOLOGICAL THEORY OF MONOCHORIONIC TWINS AS ILLUSTRATED BY A SERIES OF SUPPOSED EARLY TWIN EMBRYOS OF THE PIG

By GEORGE W. CORNER

(From the Anatomical Laboratory of The Johns Hopkins University)

The subject of identical twins is of such interest from many biological viewpoints that conjecture as to the mechanism of their origin has far outrun our actual information. Almost the whole mass of discussion now current in the literature of embryology and genetics is pure hypothesis constructed by reasoning backward from the observed anatomy of late stages, chiefly from the disposition of the fœtal membranes at birth in cases of twinning. Even the term "single-ovum twins" is an assumption, when applied to mammals.

So far as is known to the present writer the extant specimens of monochorionic twin embryos, in stages early enough to be of value for morphogenetic study, are (1) the blastodermic vesicle of the sheep, about 7 days old, and a similar embryo of the ferret (Putorius), described by Assheton ('98), in each of which two inner cell masses are found in one blastocyst; (2) the human embryo "Mateer" described by Streeter ('19a), in which a similar arrangement is supposed to exist at a slightly later stage of development; (3) two human specimens, each containing twin embryos about 12 mm. long, recently described by Arey ('22); and (4) the complete series of embryos of the nine-banded armadillo (a species in which single-ovum quadruplets are produced at every birth) which we owe to the work of Newman and Patterson ('10, '13). With Assheton's specimens and his own as the basis for hypothesis, Streeter ('19b) has constructed a very plausible series of diagrammatic stages of human single-ovum twin-formation, in which this phenomenon is represented as due to the production of two embryonic areas of inner cell masses on the inside of a single blastocyst, before the formation of the amnion. Such a hypothesis fits in well with the usual disposition of the fœtal membranes at birth; and it would also readily account for the appearance of monochorionic twinning in any mammalian species, since all mammalian embryos, whatever their later divergencies of form, pass through an essentially similar blastocyst stage. Such a view of twinning would furthermore accord with the fact of general occurrence of polyembryony in the animal kingdom, and would give added philosophical value to the experimental production of uniovular twins and double monsters in fishes and amphibians.

A different view has been proposed by Newman in his interesting book "The Biology of Twins" ('17), upon the basis of his studies of the armadillo. In this species development proceeds as in those mammals which undergo so-called "inversion of the germ-layers," until the amnion is formed; then the embryonic area divides into four embryos, and each embryo migrates down a meridian of the chorion, dragging with it the surrounding portion of the amnion, until finally a single chorion contains four embryos, each enclosed in a portion of the original amnion, now drawn out into four sacs with a common central connection. It is obvious that this form of polyembryony cannot take place except in mammals in which the formation of the embryo proper is delayed until after the appearance of the amniotic cavity; and since in man a similar proigenesis of the amnion occurs, Newman has been led to believe that in man also monochorionic twin-formation follows the same scheme as the polyembryony of the armadillo. Furthermore, if in mammals monochorionic twinning is indeed dependent upon proigenesis of the amnion, then it cannot occur in species in which the amnion is produced by rolling up of the chorion over the embryo. Newman has been willing to make this assumption, and in his book has ventured the prediction that single-ovum twins will probably never be found in ungulates.

The three specimens of early embryos of the pig which are to be described in the following pages all came into the author's hands within the space of a few weeks, at the packing house of Joseph Stern and Company, in New York City.* It is remarkable that all three were found among about 500 pregnant swine examined in this one abattoir, whereas similar specimens have never been seen in many hundreds of sows at other places. The packing-house in Baltimore which has furnished pig embryos to the Johns Hopkins laboratories for thirty years has never yielded, as far as the writer knows, anything resembling monochorionic twins except one double monster and one specimen to be mentioned later, in which it was not certain whether one or two chorions were present.

The following descriptions and illustrations will be clear to the reader who is not familiar with the specific embryology of the pig, if he will recall that the blastocyst

* The writer was at the time working under the auspices of the Department of Experimental Evolution of the Carnegie Institution of Washington, at Cold Spring Harbor, Long Island. To the Director, Dr. C. B. Davenport, thanks are due for providing support and assistance.

does not remain spherical, but is rapidly elongated to 30 or more centimeters, so that by the fifteenth day the embryo itself is a small thickening upon a very narrow but immensely elongated cylindrical chorion. The chorion does not develop villi, as does the human gestation sac, but remains smooth, and can at all times be readily peeled out of the uterus. The allantois reaches such size as to fill a large part of the cylindrical chorionic cavity, and ultimately becomes adherent to the inside of the chorion, giving rise to a diffuse allantoic placentation.

Specimen 1.—The uterus of sow C-155 contained 14 single embryos and the twins to be described. Eight of the single embryos were normal at the stage of 33-34 somites (*i. e.* of the 3rd week). Six showed abnormalities as follows: Two were retarded, without gross defects; one was retarded, with extreme flexure; one was slightly retarded, with undistended allantois. In another the embryo itself appeared normal, but its allantois was entirely cut off from the embryo by the amnion; the embryonic stump of the allantois was closed over, and the extra-amniotic part of the allantois which was adherent to the chorion had upon it a small highly vascularized vesicle. Finally, one embryo was represented merely by an almost amorphous chorionic mass. Since there were 16 corpora lutea, it seems that one of the embryos was totally missing.

In the twin specimen (Fig. 1), two embryos lay about 18 mm. apart within one continuous chorion of dimensions quite similar to those of the normal embryos in the same uterus. Fluid injected into the chorionic cavity dilated the whole space; that is, there was no septum between the embryos. When the cylindrical chorion was slit open, it was found that both embryos were abnormal as illustrated. In one, the allantois was entirely cut off from the embryo, and was growing in an almost unexpanded condition upon the chorion adjacent to the embryo. In the other, the allantois had been converted into a cluster of small irregularly dilated vesicles. The yolk-sac vessels of this embryo had not grown, as normally, out along the yolk-stalk onto the sac, but remained as a convoluted nodule on the yolk-sac near the umbilicus. That portion of the yolk-sac (or sacs) lying between the embryos had become curiously twisted and vesiculated, as shown in Fig. 1, so that it was unfortunately impossible to determine whether the two embryos had originally possessed a common yolk-sac. The two amnions seemed perfectly normal; the enclosed embryos were slightly smaller than the normal single embryos in the same uterus.

Specimen 2.—The uterus of sow C-392 contained 10 normal embryos of 11 mm. crown-rump length, and one pair of twin embryos enclosed in a single chorion (Fig. 2). There were 14 corpora lutea; three ova were therefore missing, a proportion not much above the average loss of ova in this species. The twin embryos appeared quite normal, but one of them occupied, with its allantois, about two-thirds of the available chorionic cavity, leaving but one-third for the other. The embryo with the larger allantois was 9.5 mm. crown-rump length, the embryo with the smaller allantois was 8.5 mm.; there was no significant difference in the development as indicated by external form. Injection of fluid into the allantoic cavities showed that the two were not continuous. When the chorion was opened, it appeared that the yolk-sacs, which at this stage in the development of the pig are beginning to be obliterated by pressure of the allantois, were in the twin specimen very much twisted. It was thus again impossible to determine the original relation of the yolk-sacs, for although they were entirely separate in this specimen, the separation might conceivably have been produced by torsion, as the figure clearly shows.

Specimen 3.—The uterus of sow C-66 contained 7 normal embryos of 23 mm. crown-rump length and a pair of twin embryos. There were 15 corpora lutea. Fig. 3 shows that the arrangement was somewhat different from that in the other two specimens, for here the two embryos were not placed end to end in the chorionic cavity, but side by side. The allantoides were expanded so fully that they came entirely into contact with the inner chorionic surface, obliterating the chorionic cavity. Each amnion was now surrounded by its allantois, as usual at this stage, so that each embryo with its amnion appeared to occupy a separate cavity divided from the other by a septum. The cavity was obviously the allantoic cavity and the septum was formed by the apposed walls of the two allantoides. The areas of attachment to the chorionic wall were so near together that the two umbilical cords appeared to spring from almost the same point, in the line of reflection of the allantoic septum. A section taken through this line showed clearly (Fig. 4) that the chorion was continuous over the surface, not dipping between the allantoic walls; that is, there was a single chorion containing two allantoides. Obliteration of the yolk-sacs had proceeded so far that their relation to each other could not be made out; they seemed to be separate. The embryos occupied approximately equal parts of the chorionic cavity. One was 16 mm. crown-rump length and the other 15 mm. These embryos were sufficiently advanced to permit determination of their sex by the criterion described by Spaulding ('21), which (as Dr. Spaulding assures the author) can be applied to the pig as well as to human embryos. In both these twin embryos the urethral groove runs on to the glans portion of the phallus, and they are both therefore presumably of the male sex.

Discussion

The specimens which have been described and illustrated may obviously be explained by the assumption that each is an example of twins arising within a single blastocyst, or by the alternative supposition that a deceptive appearance of twinning arose by the fusion, in each case, of two originally separate blastocysts. This latter possibility seems unlikely; one would expect to find, in addition to specimens like these, other and more numerous examples not so deceptively perfect. Such fusion is at least not common in early stages of development of the pig; the present writer has never seen it during the study of hundreds of embryos of the first three weeks. It is true that after mid-pregnancy the adjacent necrotic ends of two chorionic sacs occasionally adhere or invaginate in a more or less complete way, but in such cases the larger total dimension of the combined chorions, the considerable distance between the fœtuses, and the presence of a chorionic necrosis or invagination at the region of fusion gives sufficient evidence of the dual origin of the fœtus. In the two earlier specimens under consideration there was no evidence, to the eye or under the dissecting microscope, of any chorionic septum between the embryos, and in the third specimen there was microscopic evidence that the chorionic space was single, *i.e.*, that the septum was formed by the internal membranes alone, without participation of the chorion. On the whole, the theory that the specimens were monochorionic twins seems more

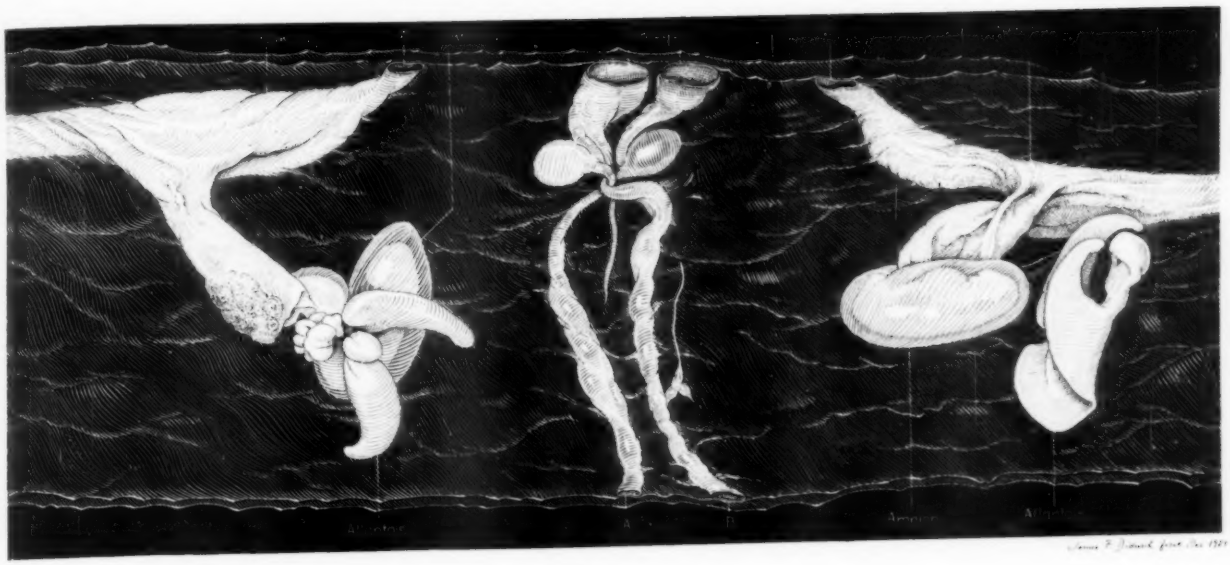


FIG. 1.

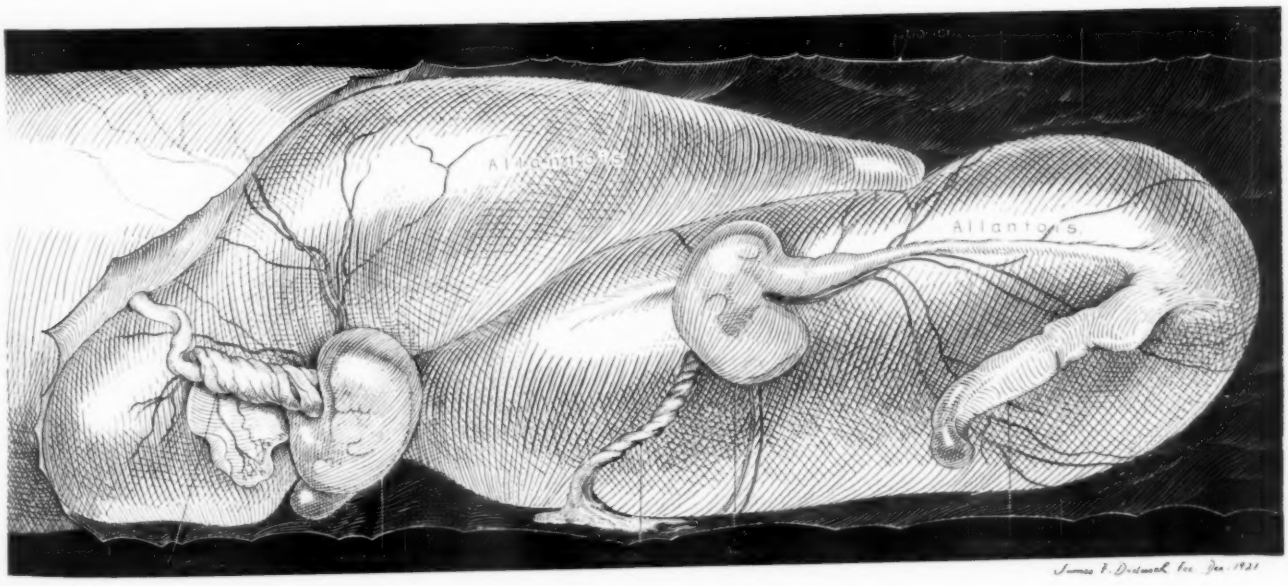


FIG. 2.

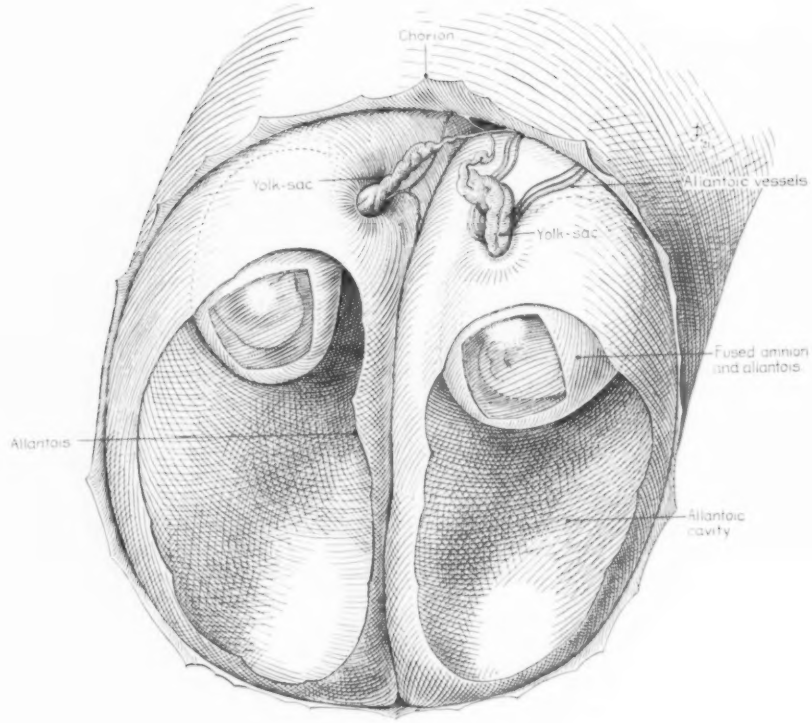

Chorion
Yolk-sac
Allantoic vessels
Yolk-sac
Fused amnion and allantois
Allantois
Allantoic cavity

FIG. 3.

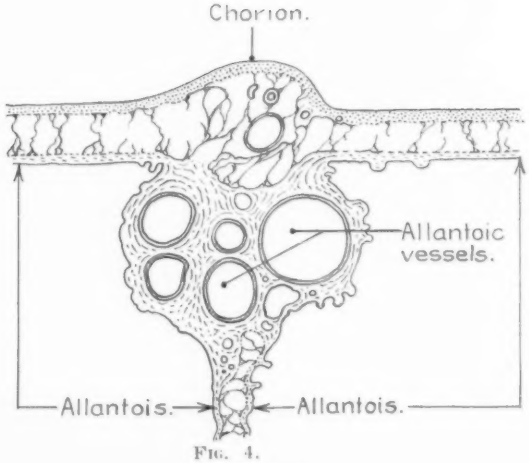

Chorion.
Allantoic vessels.
Allantois.
Allantois.

FIG. 4.

FIG. 5.

Explanation of Pig Twinning.

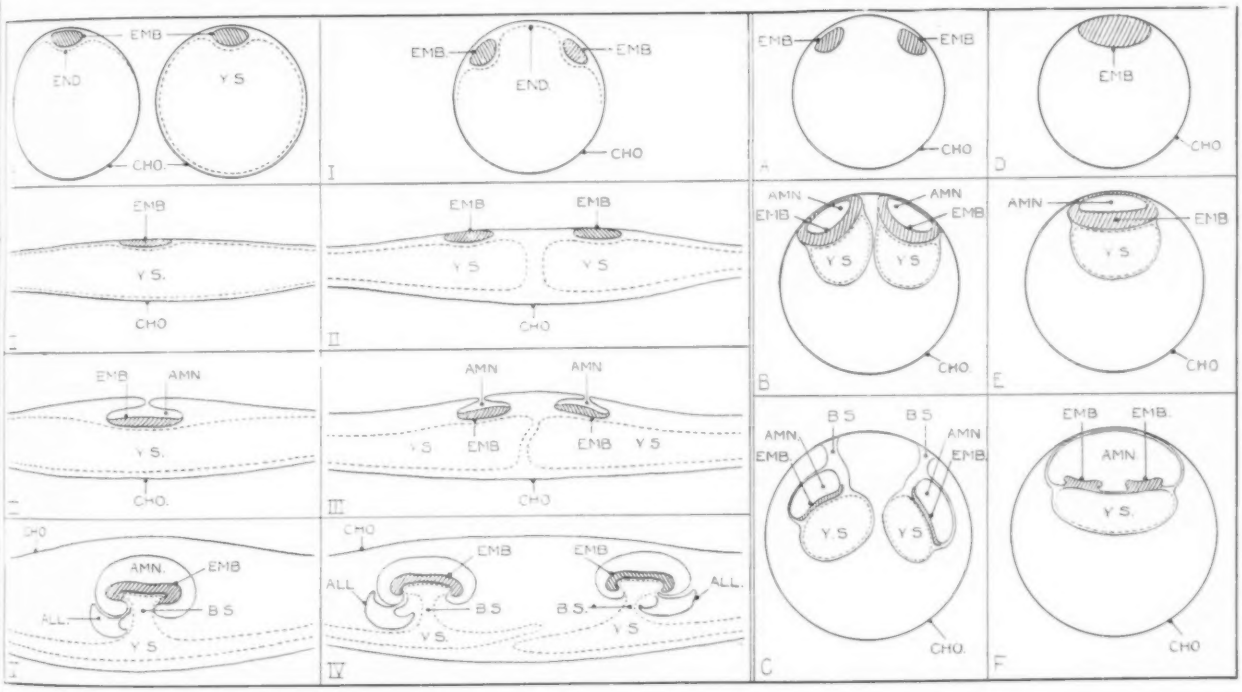
Single Twin

FIG. 6.

Hypothetical Diagrams of Human Monochorionic Twins.

Duplication before formation of amniotic cavity

Duplication after formation of amniotic cavity



probable than that they were examples of accidental fusion of separate blastocysts with obliteration of the intervening trophoblastic (chorionic) wall. It should be mentioned at this point that in a specimen from a local packing-house, studied through the courtesy of Dr. G. L. Streeter, which appeared at first sight to be another case of monochorionic twinning, in mid-pregnancy (fœtuses 130 mm. long), dissection revealed evidences hinting at a possible origin from two separate chorions, through the extensive invagination or penetration of one by the other. The impression gained from all of these specimens taken together is in accord with the recent views of Arey (1922) formulated after study of twin tubal pregnancies as recorded in the Carnegie Embryological Collection, that the appearance of monochorionic twinning may, at times be due to the fusion of two chorions, but that true polyembryony may also occur.

Assuming that our specimens are indeed monochorionic twins, it is possible to reconstruct the stages of their formation by means of a series of diagrams involving but one or two hypothetical steps. In Fig. 5, I to IV, such a series is placed in parallel with the actually known stages of development of the single pig embryo. Stage I in the twin series is borrowed from the sheep of Assheton's description, stages II and III are hypothetical, and stage IV is specimen 1 of the author's collection. In this scheme it is assumed that twinning is first made evident by the appearance of two embryonic areas (inner cell-masses) on the inner surface of a single blastocyst. Following this stage we may suppose that an amnion is formed about each embryonic area exactly as in single embryos. Meanwhile the endoderm has been spreading around the inside of the blastocyst. At the same time the blastodermic vesicle has been undergoing the great elongation which is characteristic of the pig and allied ungulate species. Whether the endoderm would usually form a single inner lining of the blastocyst, and thus provide both embryos with a single common yolk-sac, or pinch off into two yolk-sacs, is a matter for conjecture, but in preparing the accompanying diagrams the author has preferred to follow his actual specimens and to represent the yolk-sacs as doubled structures. The result, as shown in Fig. 5, stage IV, (also, Fig. 1, specimen 1) would be a single chorionic vesicle bearing separate embryos, each enclosed in its own amnion, with a yolk-sac which, for all we know at present, might be either doubled or common to both embryos. Each embryo would thereafter produce its own allantois, and when the allantoides had expanded to a moderate degree we would have the stage represented by Fig. 2 (specimen 2). Next the allantoides would expand so voluminously as to surround the amniotic membranes completely, as in Fig. 3 (specimen 3), in which we have a single chorion containing two allantoic cavities with the amnions invaginating the allantoic walls and the yolk-sacs passing out through the necks of the invaginations to be between the allantoic walls and the chorion. Finally, as the expansion of the amniotic cavities brought about complete fusion of the three fœtal membranes, the chorionic cavity would be lined at all points by both allantoic and amniotic tissue, and would be divided into two cavities by the abutting portions of the respective allantoic-amniotic membranes.

It thus seems apparent that monochorionic twinning in ungulates is not to be considered impossible merely because in mammals of this order the embryonic area is well differentiated before the amniotic cavity arises. Proigenesis of the amnion may no doubt influence the morphology of twins, but the occasional occurrence of monochorionic twins is not thereby limited to any special kinds of mammals.

In conclusion, we may permit ourselves to leave the more or less solid ground afforded by our three specimens, and to indulge in a brief speculation regarding the morphogenesis of human monochorionic twins as suggested by our studies of polyembryony in the pig. We have shown the probability that, in the pig, twinning must already be apparent before the formation of the amnion, while in the armadillo, as investigated by Newman and Paterson, the polyembryonic budding of the four embryos awaits the formation of an amniotic cavity, which is thus common to all the embryos of one ovum. It may also be pointed out that the human embryo, like all other mammalia, must first pass through a stage of simple blastocyst structure with an inner cell mass, before it proceeds to the splitting open of the amniotic cavity. It is true, as Newman points out, that at this latter stage the morphological relations are much like those at which in the armadillo multiplication of the embryonic areas begins; but it is also true that at the earlier blastocyst stage there is opportunity for twinning as in the pig, by the appearance of two inner cell masses instead of one. Our hypothesis, illustrated diagrammatically in Fig. 6, is that human single-ovum twins may be of two types. One sort of twinning, (Fig. 6, A. B. C.) arising by duplication of the inner cell mass before formation of the amniotic cavity (pig type) would typically give rise to two embryos in a single chorion with two independent amniotic cavities. The "Mateer" embryo as interpreted by Streeter illustrates this relation, and it is this scheme of twin-formation which is propounded in Streeter's contribution of 1919. A second kind of twinning, occurring by duplication of the actual embryonic areas after formation of the amniotic cavity (armadillo type), would produce two embryos within a single amnion (Fig. 6, D. E. F.). There would probably be intermediate and unclassifiable stages, because the moment of separation of the embryos might be intermediate in time, (*i. e.*, during the opening of the amniotic cavity) and also because in the first type the embryos might lie so near together that the separate amniotic cavities would fuse. Our theory thus

covers, perhaps too easily, the known variations of arrangement of the fœtal membranes of twins in pre-natal stages and at birth. (For collections of such data see O. Schulze, 1897, and Wilder, 1904). The conception that there may be a series of types of human single-ovum twins accords well with the fact that there is a rather wide variability in the degree of resemblance between so-called "identical twins;" but caution suggests that we leave these concluding notions to await the further test of actual specimens.

LEGENDS OF FIGURES

Fig. 1.—Specimen 1. Embryos of the third week, showing the cylindrical chorion cut open to expose the embryos. Points indicated by the letters A-A were adjacent before the yolk-sac was cut in opening the chorion, as were points B-B. Note abnormalities of the allantoides and yolk-sacs as described in the text. (× 7.)

Fig. 2.—Specimen 2. Normal embryos 8.5 and 9.5 mm. long, enclosed in a single chorion. (× 2.5.)

Fig. 3.—Specimen 3. Semi-diagrammatic. The embryos lie side by side within a single chorion. In this stage the amnions have invaginated the allantoides. The yolk-sacs appear to be separate, as in the previous specimens. (× 2.5.)

Fig. 4.—Diagram of a section taken through the chorion and allantoic septum near the point of attachment of the embryos of Specimen 3, showing that the chorion does not participate in the formation of the septum.

Fig. 5.—Explanation of Pig Twinning.

Single Twin

Fig. 6.—Hypothetical Diagrams of Human Monochorionic Twins.

Duplication before formation of amniotic cavity

Duplication after formation of amniotic cavity

PAPERS CITED

Arey, L.: 1922 a. Direct proof of the monozygotic origin of human identical twins. Anat. Rec., XXIII, 245.

1922 b. Chorionic fusion and augmented twinning in the human tube. Anat. Rec., XXIII, 253.

Assheton, R.: 1898. An account of a blastodermic vesicle of the sheep of the seventh day with twin germinal areas. Jour. Anat. Physiol., XXXII, 362.

Newman, H. H.: 1917. The biology of twins. University of Chicago Press.

Newman, H. H. and Patterson, J. T.: 1910. The development of the nine-banded armadillo. Jour. Morph., XXI, 359.

Patterson, J. T.: 1913. Polyembryonic development in Tatusia novemcincta. Jour. Morph., XXIV, 559.

Schultze, O.: 1897. Grundriss der Entwickelungsgeschichte des Menschen und der Säugethiere. Leipzig.

Spaulding, M. H.: 1921. The development of the external genitalia in the human embryo. Contributions to Embryology, vol. 13, Carnegie Institution of Washington, Publication No. 276, p. 67.

Streeter, G. L.: 1919 a. A human embryo (Mateer) of the presomite period. Contributions to Embryology, vol. 9, Carnegie Institution of Washington, Publication No. 272, p. 389.

1919 b. Formation of single-ovum twins. Johns Hopkins Hosp. Bull., XXX, 235.

Wilder, H. H.: 1904. Duplicate twins and double monsters. Amer. Jour. Anat., III, 387.

DEVELOPMENT AND GROWTH OF THE METANEPHROS OR PERMANENT KIDNEY IN CHICK EMBRYOS

(EIGHT TO TEN DAYS' INCUBATION)

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The story of the development of the metanephros, the organ which forms the permanent kidney in the higher mammals, occupies a voluminous literature and is based on the work of many competent observers extending over a long period of time. Herring (1900), Schreiner (1902), Stoerk (1904), Huber (1905), and Felix (1914), in their classical contributions to the subject, have given a very complete résumé of the work of previous observers whose investigations, made by means of fixed sections and reconstructions, were continued until this method could yield no further results. However, with the perfection of the tissue-culture method it became evident that by this means of approach new results might be obtained in this field. Carrel and Burrows (1910) were the first to make cultures of the kidney. They observed, after five or six days, that tubes had grown into the plasma for a short distance and that there seemed to be a lumen limited by epithelial-like cells. These tubes had, they thought, the appearance of renal tubules. Champy, in 1914, also cultivated renal tissue, but he claimed that a dedifferentiation of the entire renal anlage into an indifferent epithelium took place. In 1920 Atterbury grafted the metanephric anlage of chick embryos (6 to 7 days' incubation) in the allantois, with the result that the already formed epithelial tubules proliferated and the less differentiated nephrogenic tissue acquired an epithelial arrangement. Hematopoiesis also occurred and glomeruli developed.

In the hope of clearing up some of the controversial points in the development of the kidney, a study of the metanephros by means of cultures, as well as by fixed serial sections and spreads of living tissue, was undertaken. The results obtained in regard to the earliest stages of development of the metanephros, the anlage and early developmental stages of the renal evagination and

nephrogenic tissue, and the later development of the uriniferous tubules, confirmed those of Schreiner and Huber and therefore need no further discussion. Only the observations that differ materially from those of previous observers, such as the growth of the collecting tubules, differentiation of the anlage of the uriniferous tubules, and development of the glomerulus, together with the formation of the blood-vascular system, will be discussed in this paper.

I am indebted to Dr. and Mrs. Warren H. Lewis, not only for advice and assistance, but also for the technique employed, which made possible the cultivation and observation of the renal tissue.

MATERIAL AND METHODS

Cultures were made by explanting small pieces of chick embryos after from 8 to 10 days' incubation. The pieces to be explanted were chosen with the aid of the dissecting microscope from the most inferior medial pole of the mesonephros and the superior medial pole of the metanephros, because, as is commonly known, the differentiation of the mesonephros proceeds antero-posteriorly, whereas in the metanephros it is postero-anteriorly. For this reason the degree of differentiation at any age depends to some extent on the part of the renal body from which the specimen is taken. The areas chosen were the least differentiated embryologically and the most isolated anatomically. The tissue was cut up into pieces as thin as possible and placed on sterile coverslips, in a small drop of Locke-Lewis solution (85 c.c. Locke's solution plus 15 c.c. chicken bouillon plus 0.5% dextrose). The coverslip was then inverted so as to form a hanging drop, sealed onto a ring of vaseline, and incubated at 39° C. The cultures were observed at varying intervals in a warm box at 39° C. Some of them, after varying periods of growth, were fixed either by means of iodine vapor or of Zenker's fluid and stained. Serial paraffin sections 5 micra in thickness were cut, stained with hematoxylin-eosin, and mounted separately. The entire metanephric body of both injected and non-injected chicks was studied at different ages in fresh spreads.

MORPHOLOGY

The urino-genital system of chick embryos from 6 to 10 days' incubation can be dissected with considerable ease without the microscope. When the abdominal cavity is opened and the stomach and intestines have been removed, the mesonephric bodies appear as two greenish-red structures filling up a large part of the remaining space in the abdomen. They are situated one on each side of the vertebral column and extend from just below the attachment of the liver to a point immediately above the bifurcation of the abdominal aorta. They are more or less bilaterally symmetrical and seem to be connected across the mid-line by only a thin sheet of mesothelium. Their dimensions vary according to the age of the embryo; between 6 and 10 days' incubation they average about 1 mm. in diameter and about 3 mm. in length. They are somewhat wedge-shaped pyramidal bodies, diminishing in width toward the caudal pole. The color is characteristic and is in marked contrast to that of the metanephroi and other abdominal viscera. Over the glistening surface can be seen many pin-point red spots, the glomeruli. At the upper (cephalic) poles are the gonads, two opague white tubular-like bodies, which, starting above and behind the pointed upper poles of the mesonephric bodies, continue across the uppermost anterior surfaces and course caudalward along the antero-medial margins, ending in a point corresponding to one-half the length of the mesonephric bodies. From the dorsal surface, and continuing below the lower pole of each mesonephric body, is the mesonephric or Wolffian duct, which serves as the excretory duct for the mesonephric body or embryonic kidney. These small white ducts approximate each other in a downward course and end in the cloaca. The branching connections of the Wolffian duct with the mesonephros, the mesonephric ureters, could be distinctly seen by elevating the lateral margins of the mesonephric bodies. Along the vertebral column, dorsal and also immediately posterior to the mesonephric bodies, lie two bilaterally symmetrical translucent structures, the metanephroi. The genito-urinary apparatus is entirely covered by a thin layer of cubical epithelium which is known as mesothelium or cœlomic epithelium. In the youngest ages, the metanephros is completely obscured by the overlying mesonephros, which reaches its greatest volume at about the eighth day. From this time on, however, the metanephros increases rapidly in size, with the result that in a 10-day embryo the mesonephros appears to be a relatively small body situated on the anterior surface of the metanephros. The supply of blood to the metanephric body between these ages is very small, while the blood-flow through the mesonephros is quite abundant, a fact which accounts for the difference in the color of the two bodies. The shape of the metanephros changes constantly during the growth and development of the embryo, so that no definite shape can be described as typical for all ages. However, in general it may be said that the shape approximates that of a dumb-bell, being broad at both poles and narrow in the middle. This is well shown for older embryos (18 days) by Minoura (1921, Fig. 8). At the age of 6 days the metanephric body has a smooth exterior surface, with no lobulations. A little later, however, the bodies begin to show definite lobulations, which by the tenth day have become discrete lobes completely covered by cœlomic epithelium.

The entire metanephros was dissected out and placed on a slide, thus making it possible to study the fresh tissue at different ages with the aid of the microscope.

In the 6-day embryo it was found that the metanephric tubule, which sprouts off the Wolffian duct, had already grown into the metanephric body, completely traversing the undifferentiated tissue from the posterior to the anterior pole. Along the entire length of the metanephric tubule the primary collecting tubules had budded off in three main planes, lateral, dorsal, and ventral, and there seemed to be an enlargement of the lumen of the metanephric tubule at the anterior and posterior poles, at which places the metanephric body is broader. Each of the primary collecting tubules, although they had grown only a slight distance from the metanephric tubule, had just divided dichotomously into two secondary collecting tubules. No further division, that is, beyond the secondary tubules, was ever observed at this age. Immediately about the primary and secondary collecting tubules was a sharply defined covering, consisting of a single layer of endothelial cells. This structure will be described in detail later. Between the bases of the primary collecting tubules, near the posterior pole of the metanephric body, sinuses had differentiated out of the tissue surrounding the bases of these primary collecting tubules. These spaces or sinuses were lined with flat endothelial cells which gave rise to blood elements. This was the first appearance of sinus formation observed in the metanephrogenic body.

At 7 days the branches of the primary collecting tubules, namely, the secondary tubules, had again divided dichotomously into tertiary tubules. The branching appeared always to occur in a forked manner, the tubules growing toward the periphery. There was a marked increase in the length of the primary and secondary collecting tubules during the sprouting of the tertiary tubules. The apparent dilatation of the lumen of the metanephric tubule at the anterior and posterior poles had disappeared by this time, but in these areas there was a much more rapid growth of the collecting tubules than in the intermediate region. This growth was perhaps somewhat more pronounced at the posterior than at the anterior pole, for, as is well known, the metanephros differentiates postero-anteriorly. In the body of the metanephros the formation of sinuses proceeded hand-in-hand with the elaboration of the collecting-tubule tree, replacing the undifferentiated tissue about the bases of the primary and secondary collecting tubules. Towards the periphery of the metanephric body, strands of mesenchymal cells, arranged in festoons, divided the cortex into a series of lobes which corresponded roughly to the secondary collecting tubules. The definition of this lobulation was more pronounced in the 8-day embryo, constituting complete separation of the metanephrogenic lobules into distinct lobes surrounded entirely by a layer of cubical cells and arranged always in relation to the secondary collecting tubules. The length of the primary, secondary, and tertiary tubules increased greatly with this lobulation, the tertiary tubules being entirely intralobar, while the primary, as well as the secondary tubules, were extralobar. In the anterior and posterior poles of the metanephros the tubules grew to a greater length and gave off more sprouts, resulting in a greater number of secondary and tertiary branches in these regions. As has been stated above, the lobes were established in the 8-day embryo, but an intralobar lobulation was observed to begin immediately following the lobe formation. This subdivision of the lobe into smaller lobules persisted until the elaboration of the collecting tubule tree was complete. In the 9-day embryo it corresponded to the branching of the quarternary tubules or the first intralobar division of the tertiary tubules. Up to this time no sign of the secreting or convoluted tubule had appeared. About these quarternary tubules, however, differentiation of the future convoluted tubule had occurred and from now on, with each succeeding division of collecting tubules, there was a simultaneous formation of convoluted tubules (including glomeruli, etc.) in the angles of the branching collecting tubules. Eventually, each branch of the collecting-tubule tree, with its corresponding convoluted tubules, was surrounded by mesenchymal tissue which formed a sort of capsule about it as a unit. In the kidney, therefore, as in the lung, liver, etc., there is a definitive lobule, which is the elementary unit of the entire excretory apparatus. This unit consists of the terminal branch of the collecting tubule, the convoluted tubules, including the glomeruli, and the surrounding endothelial and mesenchymal tissues. Owing to the lobulation, the growth and division of the collecting-tubule tree inside the lobe was much more elaborate in the 10-day than in the 9-day embryo. The distribution of the lobes in the 10-day embryo corresponded exactly to the distribution of the secondary collecting tubules. As a result, the greatest number of lobes were found composing the caudal pole, while the next greatest number constituted the cephalic pole. The lobes making up the intermediate region were evenly and regularly distributed in all planes, so that all the lobes at any given level were practically at the same stage of development regardless of their distance from the original metanephric tubule. Differentiation of the lobe always proceeded from the base towards the periphery. The most developed convoluted tubules were associated with the quarternary branches of the metanephric tubule. From this base or center the cycle of development was repeated for each generation of the renal units as we approach the periphery of the lobe towards the cortex and also towards the future columns of Bertini, not only in the longitudinal diameter but also in the transverse diameter. The development of the systems of sinuses obeyed the same general principle, that is to say, they were formed first at the base of the lobe and later at the periphery.

In conclusion, it may be stated that, although the posterior portion of the metanephric body grew and developed more rapidly than the anterior portion, nevertheless differentiation was always from the metanephric duct towards the periphery, not only in the lobes and lobules but also in the blood-vascular system and in the supporting tissue; so that even in the older embryos the most mature elements were located near the base of the lobe, while the immature structures occupied the cortical regions.

GROWTH AND DEVELOPMENT WITHIN THE EXPLANT

In cultures of chick embryos of from 8 to 10 days' incubation the evolution of all the elements making up the excretory unit of the permanent kidney could be followed in detail throughout their differentiation and development. Usually the explants contained at least one lobe with its lobules, in different stages of development according to the age of the embryo from which the piece was taken. Observations on the mesonephros were made for a comparison, but these will be described only where they differ in some fundamental way from those made upon the metanephros. The component parts of the excretory unit in cultures of different ages will be described in the order of their differentiation and development: *i. e.*, collecting tubules, nephrogenous tissue (secreting or convoluted tubules and glomeruli), and endothelium, including the blood-vascular system. The growth and development within the explant, as well as the growth extending out from the explant, were studied.

EIGHT-DAY EMBRYOS

The living explant was slightly yellowish and so transparent that the structure of the metanephros could be readily observed. The most striking characteristic of the explant was the collecting tubules, which at this age have undergone extensive growth from the metanephric ureter into the undifferentiated metanephrogenic tissue and become branched like a tree, as was mentioned when speaking of the morphology. It is in the growth of this element of the excretory unit that the mesonephros and metanephros differ most widely. In the mesonephros the collecting tubule, from the beginning of its growth from the primary excretory duct, is a single unbranched tubule, twisted upon itself, opening individually into the excretory duct, and destined to become connected with one convoluted tubule. It is coiled from the beginning, and becomes more so as it approximates maturity. The characteristic tree-like formation observed in the metanephros was never found in the mesonephros.

In the explants from 8-day embryos masses of undifferentiated metanephrogenic tissue surrounded the ends of the collecting tubules and, as there were no other differentiated elements of the excretory unit, the collecting-tubule tree (primary, secondary, and tertiary tubules) stood out strikingly because of the marked contrast between the differentiated and undifferentiated tissue (Fig. 1). After the culture had grown for 20 hours, the explant became flattened out and attached to the coverslip, owing to the migration of connective-tissue cells, nerve filaments, endothelium, and tubules. Coincident with the migration was a division of these cells, thus forming an extensive area of new marginal growth (Figs. 3, 13, and 14). The migration of cells from within the explant to form the marginal growth left the collecting tubule intact and sharply defined (Figs. 2 and 3). The definition was aided by a single layer of highly refractive endothelial cells which completely enshrouded each collecting tubule and its branches, even covering the terminal ends of the tube (Figs. 2, 6, and 18). The endothelial cells formed a very thin sheet, through which the bases of the columnar cells forming the tubule could be seen as triangular or prismatic cellular bodies. The collecting tubules grew in a comparatively straight line from the tertiary tubules and branched out in a characteristic and regular manner, *i. e.*, always from the base towards the periphery of the lobe (Figs. 1, 2, and 8). There may be a slight, gentle curvature to the course of the growth of either the main trunk or its branches, but never was there seen the bending and intertwining observed in the tubules of the mesonephros or later in the convoluted tubles. There was a regular gradation in diameter from the tubules making up the central trunk to those constituting the peripheral arborization. The distance between the branches along the main stem was the same, although the branches came off in any plane (Fig. 2). The most common type of branch formation was dichotomous or trichotomous (Fig. 1), although there was occasionally a branching into fours. The branches always came off from the main stem at angles greater than ninety degrees, and grew in a lateral or peripheral direction. However, it must be stated that, while there was no constant direct trunk or axis of the tree growing through the center of the lobe with the branches coming off laterally, yet there was always one main branch, which maintained a general peripheralward course of growth and remained approximately the axis of the lobe (Fig. 2). The lumen of the collecting tubule was established at this age and patency was maintained by a fluid-like medium in which granules and cellular detritus were seen floating about. The lumen was bordered by a high, non-ciliated, columnar epithelium (Figs. 12 and 24). About the ends of the tubules the undifferentiated metanephrogenic tissue was observed in the form of a cap of oval cells arranged radially. These were packed together quite closely, forming a layer about four cells thick, which was, as a general rule, more dense and thick immediately over the ends of the tubules and tapered around all sides of the tubules for a short distance. Peripheral to this cap was another layer of undif-

ferentiated metanephrogenic tissue, the cells of which were less densely packed, contained fewer granules, and had no characteristic arrangement. These two regions were first described by Schreiner (1902) as the inner and outer zones, respectively, of the metanephrogenic tissue. Beyond this was undifferentiated mesenchyma, which followed the same arrangement as the cap and, by dipping down between the ends of the branches of the collecting tubules, marked out what would eventually be the lobulation of the permanent kidney (Fig. 2).

In cultures 48 to 144 hours old, the explant had become so transparent that the actual growth of the collecting tubule was easily followed. This growth took place not only by mitotic division of the cells all along the length of the tubule, but also from the ends of the tubule, the latter being the more active of the two and the more effective in increasing the length of the tubule. In the end of the tubule in which growth and branching were about to take place, there occurred a symmetrical bulbous or ampullar formation, due to a general proliferation and change in shape of the cells making up the the ends of the tubule. In areas of active proliferation and rapid growth, there seemed to be a metamorphosis always to the spherical type of cells with a subsequent reversion to the adult type. As a result of this proliferation, there occurred, coincidentally with the bulbous expansion, bud-like projections from the blind end of the tubule. Usually these projections were situated at opposite poles of the tubule; occasionally there were three or even four of them. Their rate of growth was approximately the same. At first they appeared to be growing out horizontally to the cortical surface of the lobule; they soon altered their course, however, one growing almost perpendicularly and continuing in a general way the peripheralward growth of the tree, while the other or others tended to grow out more horizontally, making up the surrounding arborization. These bud-like projections were at first solid sprouts, but by continued proliferation and reshaping of the spherical cells into a columnar type the bud increased in length, breadth, and thickness and by a rearrangement of the cells a lumen was formed, which in a very short space of time extended almost the entire length of the sprout; thereafter, the lumen formation progressed hand-in-hand with the growth of the tubule. This phenomenon was of a different type from that described by Dr. Sabin (1920) in the formation of the lumen of the blood-vessels, for in the collecting tubule no vacuolization of the cells took place; on the other hand, a small but gradually widening cleft within the sprout, was formed by a rearrangement of the cells and a transformation in shape to that of the columnar type. The new collecting tubule branches continued to grow with a simultaneous formation of a lumen, until a certain length had been attained, when a new branching occurred. The endothelial sheet covering the tubule proliferated and grew simultaneously with the growth of the branches, and the collecting-tubule tree was thus covered throughout with a shroud of endothelium. The undifferentiated metanephrogenic tissue was plainly visible in the cultures and its growth and development were closely associated with that of the collecting-tubule tree. Felix (1912) described this as forming a circumferential sheet which surrounded the ends of the ingrowing collecting tubules and was pushed peripheralward by the growth of the tubules after each succeeding layer of newly formed convoluted tubules and glomeruli had been formed. This, however, was not found to occur in the chick. Here the undifferentiated metanephrogenic tissue surrounded each individual collecting tubule that grew into the lobe; as the tubule branched, this metanephrogenic tissue was split up, some of it being carried forward by the growing branches, the rest remaining behind in the angles between the branches. The portion of the metanephrogenic tissue carried forward over the end of the tubule extended to cover the new branches of the tubule, partly by proliferation and partly by a peripheralward migration, as was evidenced by a slight distortion of the cellular mass.

The deposition of the undifferentiated tissue in the angles of the branching tubules was found not only in the cultures and spreads but also in the sections of the metanephros (Figs. 10, 13 and 16), and it was from this tissue that the future convoluted tubules and glomeruli always developed. This constant relation of the undifferentiated metanephrogenic tissue to the ingrowing collecting tubule was especially striking in the cultures. No matter to what extent the collecting tubule may have grown inside the explant or out into the margin, as will be shown later, there was always the same relation maintained between it and the undifferentiated metanephrogenic tissue (Figs. 8 and 10). Since the convoluted tubules always developed in the same position with relation to the collecting tubules, and always from the metanephrogenic tissue of the inner zone and never elsewhere in the mass of undifferentiated tissue, it may, I think, be deducted that the metanephrogenic tissue of the inner zone is made up of cells that are predestined to become the anlage of these convoluted tubules. This anlage always appeared as a mass of undifferentiated tissue, quite distinct from the surrounding nephrogenous tissue of the outer zone and the collecting tubule (Figs. 10 and 15). The cells constituting the inner zone, which formed a cap-like covering over the blind end of the ingrowing collecting tubule, were at first quite similar in form. However, within 24 hours, in cultures of the 8-day embryos, morphological changes, and also changes in the general arrangement of the cells, were observed. The cap became sharply defined by three and often four layers of cells which completely ensheathed it (Figs. 15 and 18). The cells making up these layers were rather long and narrow, approximating the endothelial

cell in shape, and stood out in marked contrast to the adjacent tissue. Between the two outermost layers appeared a space, which likewise encircled the anlage and served to define it still more sharply (Figs. 16, 18, and 24). Simultaneously with the differentiation of this endothelial sheath, there occurred not only a change in the morphology of the cells making up the cap but also a change in the form of the cap itself. The cells proliferated rapidly, becoming more spherical in shape and somewhat less granular. There was also an increase in the size of the individual cells. Thus there was formed, as it were, a solid central core of spherical cells approximating the epithelial type (Figs. 15, 16, 18, and 19). The difference in the polarity of these two distinct types of cells was quite striking. In the spherical cells there was a tendency toward a radial arrangement about a central core. The axes of these cells were perpendicular to the axis of the central core, while the axes of the endothelial-like cells about the periphery of the central core were at right angles to the axes of the spherical cells (Figs. 16, and 18). In twenty-four hours' growth, before any suggestion of tubule formation, there was thus differentiated *in situ* two distinct types of cells, endothelial and epithelial, from one common mass of undifferentiated tissue. Furthermore, the entire anlage had become surrounded by a space lined with endothelium (Fig. 18).

Concomitantly with the differentiation of these two types of cells, the inner cell mass, composed of spherical epithelial-like cells, was observed to become shaped like an inverted comma (Figs. 19 and 20). (This comma shape was first mentioned by Ribbert in 1899.) The head of the comma was always to the side of the ingrowing collecting tubule, while the tail covered its blind end (Figs. 16 and 19). By continued growth the comma-shaped mass increased in size and underwent a gradual transition into a solid S-shaped tubule or core. This transition required about 24 hours. At first its differentiation and growth were uniform throughout, that is, the tail end of the comma appeared quite as early and was as far advanced as the head-pole (Fig. 19). However, during the transition the most rapid growth was seen to occur in the head pole of the comma, or what I shall call the distal pole, where the spherical cells became arranged in what appeared to be a solid, sphere-like dilatation (Fig. 21). Immediately above this sphere the growth was almost as rapid, causing an angulation which resulted in the formation of the first curve of the future S-shaped tubule (Fig. 21).

Differentiation beyond this stage was not observed in the metanephrogenic tissue of the inner zone in the 8-day embryos. In the outer zone, however, growth and differentiation did occur. The future connective-tissue septa appeared at the periphery of this outer zone of the metanephrogenic tissue and with their appearance there were laid down the interlobular septa constituting the microscopic lobulation. Throughout the growth of the collecting-tubule tree, lobulation was observed to keep pace with the formation of branches of the tree.

Nothing has yet been said concerning the region about the main stems and branching collecting tubules. Concomitant with the growth and development of the lobule, there was constantly left behind, in the path of differentiation and peripheralward growth, a cellular mass of tissue which surrounded the trunk of the collecting-tubule tree for that lobule. This tissue constituted a supporting framework containing the blood capillaries, for the developing excretory unit. Simultaneously with the differentiation of the excretory unit, and well advanced before the appearance of the convoluted tubules, there was formed progressively a system of sinuses. As stated under the description of the morphology, the process of differentiation and development of endothelial sinuses was first observed in the undifferentiated metanephrogenic tissue between the bases of the primary collecting tubules in the 6-day embryo. The presence of a sinusoidal circulation in the mesonephros was first mentioned by Minot (1892), but nothing was said about the existence of a sinusoidal circulation in the metanephros or the method of development of these sinuses. In cultures of the 8-day embryo all stages of development of the sinuses could be seen. The most fully developed were near the base of the lobe, while the least developed ones were found about the anlage of the excretory unit, toward the periphery. This formation of sinuses followed, so to speak, in the wake of the growing collecting-tubule tree (Figs. 1, 13, 14) and, as in the differentiation and development of all the other elements of the lobe, the progression was always from base to periphery. In the cultures the sinuses were sharply defined from the surrounding tissue and they also were readily seen in fixed sections, although much shrunken and distorted (Fig. 16). About the base of the lobes the sinuses were quite large and filled with a fluid medium, in which blood elements were commonly found (Figs. 5, 13, and 17). The walls of the sinuses consisted of a single layer of endothelial cells, which at times were in apposition to the endothelial layers covering the tubules, and at other times were connected with these layers of endothelium by endothelial sprigs or offshoots (Figs. 12, 16, 17, 24). The larger sinuses connected freely with each other and also with a capillary network of the lobe, so that in an embryo of this age there were two distinct blood-vascular systems, capillary and sinusoidal. Blood-islands were commonly seen attached to the inside of the walls of the sinuses and from these hemoglobin-containing cells, as well as white blood-cells of lymphocyte type, were differentiated. Sinuses also were differentiated *in situ* from the undifferentiated tissue making up the bulk of the metanephric body. These sinuses had their origin in the vacuolization of cells quite similar to angioblasts. These cells were rather

conspicuous, due to the fact that there were always two and sometimes three in juxta-position, making them appear as deeply pigmented multinucleated giant cells, joined together by very minute protoplasmic processes (Fig. 6). In each cell there appeared a vacuole, which constantly enlarged until there remained only a rim of cytoplasm and an eccentrically placed nucleus. The vacuolization of these cells was complete in 24 hours and fusion of the two or three original cells occurred, resulting in a small space surrounded by flattened out cytoplasm and eccentrically placed nuclei. This space increased in size by multiplication of the flattened-out cells and was held patent by a fluid medium. These sinuses were of different shapes and sizes and were connected with each other, as well as with the capillary network, and with the endothelial spaces about the anlage of the convoluted tubule, by means of direct sprouting of the larger sinuses. Throughout the entire life of the culture differentiation of capillaries *in situ* and direct growth after differentiation by sprouting was observed. Thus, the sinusoidal and the capillary systems develop *in situ* at the same time from a totally undifferentiated mass of tissue making up the bulk of the metanephric body. The two types of circulation were very similar in their differentiation and development.

In old cultures the macrophages appeared to ingest and digest tissue left behind in the wake of the developing lobe (Fig. 14) as well as injured tissue. Inside these macrophages red blood-cells and other forms of cellular detritus were observed. The macrophages were much more numerous in the base of the lobe.

Nine- and Ten-day Embryos

In the explants of the metanephros, made from 9- and 10-day embryos, one had, of course, a more completely developed excretory unit to begin with than in the explants from 8-day embryos. Notwithstanding the fact that there were growth and differentiation in cultures from the latter, these were always much retarded as compared with an embryo of corresponding age. In the explant of the metanephros from 9- and 10-day embryos, the collecting-tubule tree had become much more branched and complex throughout the lobe. The main trunk had increased not only in length but also in diameter, and its primary branches had already divided into secondary, tertiary, and quarternary branches. The dichotomous branching of these tubules continued with a lobulation or division of the lobe into smaller lobules by the rearrangement of the mesenchymal septa, corresponding to the branching tubule tree. The sinuses and blood-vascular elements formed and grew just as in cultures from the 8-day embryo, so that the essential difference between the growth of the explants in the two series lay in the differentiation and development of the secreting tubules.

All stages, from completely differentiated to entirely undifferentiated metanephrogenic tissue, could be studied in the explants of 9-day embryos, as there was constantly occurring a repetition of the cycle of differentiation and growth of the complete excretory unit towards and in the future cortical portion of the lobules. Between the 9th and 10th day of incubation seemed to be the optimum age for a study of the development of the metanephros. Whether this was due to the fact that metanephrogenic tissue was of a greater vitality than at 7 and 8 days, or whether the undifferentiated cells were more nearly on the verge of differentiation and therefore less affected by the change in environment, I cannot say; in any event, within twenty-four hours all stages of differentiation from a comma-shaped body to an S-shaped tubule could be observed. The main areas of growth during the transition were the sphere-like group of cells at the distal pole and, immediately superior to this, the portion which was to form the first knuckle of the future S-shaped tubule (Fig. 21). At these two points proliferation was most active. The continued growth of the sphere at the distal pole, immediately under the rapidly proliferating first curve, caused a slight flattening of the sphere with a subsequent bulging about the sides and to some extent the ends of the overhanging knuckle of the first-formed curve. The growth of the sphere and that of the large knuckle (first curve), being parallel and in the same direction, caused an acute flexion of the solid core immediately posterior to the sphere, thus forming the second curve of the S (Fig. 22). Owing partly to the relatively slow growth of the cells making up this second curve, and partly to the pull of the rapidly growing sphere and first curve, the second curve became very small in diameter and acutely flexed (Figs. 6, 23). The solid S-shaped mass, therefore, developed *in situ* and not, as heretofore thought, from a sphere into an S-shaped tubule. The entire solid core developed from cells already present, and assumed an S-shape because of more rapid growth in some regions than in others.

The endothelial sheath, together with the sinus formation, progressed hand-in-hand with the development of the S-shaped core, i. e., the S-shaped core was completely covered by a layer of endothelial cells and in addition to this the entire anlage was completely surrounded and delimited by a space or sinus lined with endothelium (Figs. 12, 16, 24). The core was thus sharply outlined. A marked difference in the refractivity of the endothelial and epithelial cells further aided in the definition of the tubule so that it was easy to distinguish changes taking place within the tubule from those in the tissue without. The close approximation of the glomerular pole to the under surface of the first curve of the S might give the false impression of a cleft formation, as stated by Ribbert (1899) and Huber (1905) instead of a solid core doubled upon itself (Figs. 21, 22). The potential space between the distal pole and the under surface of the first

curve contained the endothelial layers covering the solid tubule and those forming the wall of the sinus (Figs. 17, 21, 24), so that the bud of an endothelial sac was thus placed between the glomerular end of the tubule and the under surface of the first curve. This slight indentation, however, approximated in no way the cup formation described by other investigators, notably Huber, Schreiner, and Felix.

After the comma-pattern had developed into an S-shaped tubule, there was a continued growth throughout the entire tubule. Proliferation of the cells in the glomerular end occurred more rapidly than elsewhere in the S-shaped tubule until that end became dilated and bulbous, forming a tuft of epithelial cells (Figs. 2, 6, 11, 22). At this stage no capillaries or blood elements were ever observed in this tuft, all the cellular proliferation being inside the endothelial sheath, and therefore within the tubule. The cells causing the dilatation of the glomerular end were at first all more or less round; however, a differentiation of these cells took place *in situ* along with their continued proliferation, resulting in the formation of flat, endothelial-like cells, and spherical epithelial cells, thus constituting the pre-glomerular tuft (Figs. 17, 23). The endothelial cells, because of their shape and greater refractivity, could be traced winding about amongst the epithelial cells (Figs. 23, 24). The latter, however, formed the greater part of the cell mass producing the dilatation of the glomerular end of the tubule. By 72 to 96 hours, collapsed endothelial spaces could be seen winding through the epithelial tuft and everywhere covered with epithelial cells (Fig. 24). Thus the presence of endothelial cells intimately intermingled with epithelial cells was observed in the bulbous glomerular end while the future secreting tubule was still in a solid state and as yet no connection with any type of circulatory system could be seen. No evidence of a growth from the endothelium covering the exterior of the tubule down into the glomerular end was observed. The cellular proliferation in the glomerular end proceeded rapidly, the dilated bulbous end being completely filled and much distended in comparison with the remainder of the tubule. The curve of the tubule immediately proximal to the glomerular end became reduced to a very small tube whose walls were formed by a rather low cuboidal epithelium one cell in thickness, covered by an endothelial sheath. In the loop of the S immediately proximal to the glomerular end the formation of a lumen was first observed (Fig. 23). This followed the same course as in the collecting tubules, *i. e.*, the lumen formed by a rearrangement and separation of the cells and not by vacuolization, as was observed in the vascular system.

The cells abutting against the lumen were the first ones to become cuboidal in shape. The rearrangement of the cells about the lumen progressed distally and proximally with about equal rapidity. The lumen formation began about the inferior half of the spherical tuft of cells, then progressed to the superior half, until the central tuft was completely separated from the walls of the glomerular end of the convoluted tubule, except at one pole, usually distal to the point of transformation of the secreting tubule into the glomerular end (Figs. 6, 23, 24).

This lumen formation in the glomerular end was the anlage of the capsule of Bowman, the cells bordering the lumen constituting the layers of the capsule (Fig. 24). The flat epithelial cells formed the parietal layer, while the cuboidal cells made up the visceral layer. Immediately before the formation of the lumen the spherical tuft of cells attained its maximum size, decreasing from that time on until the glomerulus began to function, when it again enlarged. With the completion of the lumen formation and the changes in shape of the bordering cells forming the anlage of Bowman's capsule, the appearance of an invagination of the remainder of the spherical tuft was obtained. However, the etched-like outline of endothelial cells enabled one always to separate that which occurred inside the tubule from that which occurred outside. Any invagination or cup formation that involved the entire glomerular end of the secreting tubule would of necessity cause a disturbance of the contour outlined by the ensheathing endothelial covering. If sections of merely the cuboidal cell layer of Bowman's capsule constituting the most distal margins of the glomerular end of the tubule are reconstructed, then one obtains an incorrect impression of a tubular invagination.

A connection was now established between the newly formed space or sinus surrounding the secreting tubule and the larger sinuses already formed. This connection was by means of direct sprouting off of endothelial strands from the larger sinuses which became confluent with the sinuses differentiated *in situ* about the anlage of the secreting tubule. Thus the endothelium-lined space between the superior surface of the glomerular end and the second curve of the S-shaped tubule was in direct communication with the larger sinuses. A continuation of endothelium was then established from the endothelial elements within the glomerular epithelial tuft through the sinus about the secreting tubule by way of endothelial sprouts into the larger sinuses about the collecting tubules (Figs. 2, 24). The endothelial sprouts connected the glomerulus with the sinuses always through the attachment of the glomerular tuft of cells to the wall of the tubule; that is, at the point where the capsule of Bowman was reflected, which usually lay distal to the urinary pole. At this point the visceral and parietal layers of the capsule were confluent. In the older cultures it was found that the sinuses became connected with the capillaries that developed in their neighborhood.

Hematopoiesis occurred in the spherical tuft of the glomerular end with the formation of red blood-cells, plasma, and white blood-cells having the appearance of

lymphocytes (Figs. 17, 23, 24). The formation of the blood-elements was usually first noted in that portion of the tuft adjoining the secreting tubule and progressed from this point throughout the glomerular portion. It occurred at about the same time the connection with the sinuses took place. It must be borne in mind, however, that in the cultures no circulation was present. At no other point in the entire excretory tubule did hematopoiesis occur, a fact which also indicates the endothelial nature of the cells inside the spherical tuft Lumen formation by thinning out of the cells, characteristic of the blood-vascular system elsewhere, was found to obtain in the glomerular tufts. The blood elements made the definition of the endothelial channels much more pronounced and the latter could be traced with ease, winding among the epithelial cells and covered by a single layer of these cells.

After the differentiation, the convoluted tubule continued to grow in exactly the same manner as the collecting tubule, that is, by proliferation of the cells along the entire tubule or at the end nearest the collecting tubule. The convoluted tubule grew always toward the collecting tubule, until finally the proximal end of the former abutted against the side wall of the latter. This contact usually, though not always, was in the immediate region of a newly formed branch of the collecting tubule. The anastomosis of the convoluted and collecting tubules could be observed in cultures of the 9-day embryos after 24 hours' growth. In general, it required about that length of time to effect complete continuity of the convoluted and collecting tubule lumina. The continued growth of the secreting tubule against the wall of the collecting tubule exerted sufficient pressure to indent the latter and cause a distortion of its constituent cells (Fig. 23). The secreting tubule grew into the collecting tubule at an angle of about forty-five degrees. The cells in the wall of the collecting tubule formed a sort of arciform arrangement about the ingrowing blind end of the convoluted tubule. There was a rapid proliferation of the cells constituting the latter and the cells reverted to a spherical type, as was observed in the growth of the collecting tubule. As a result of this proliferation, the end of the convoluted tubule protruded like a wedge through the wall of the collecting tubule into the lumen. This was not due merely to pressure from the outside against the wall of the collecting tube; there was an actual growth of the convoluted tube inside the wall of the collecting tube, the cells dividing and making room for the increased number. An interruption in the continuity of the wall of the collecting tubule was thus effected (Fig. 24).

Lumen formation in the secreting tubule followed upon the proliferation of the proximal or blind end, so that about two hours after the secreting tubule had grown through the wall of the collecting tubule the lumina of the two had become continuous (Fig. 24). This continuity was proved by the coursing of fluid containing granules back and forth from one tubule into the other. After the anastomosis of the convoluted and collecting tubules, there was continued growth of the former throughout its entire length, as evidenced by mitotic figures. The greatest growth activity, however, occurred in the convoluted portion next to the collecting tubule, which in the adult kidney would correspond to the distal pars convoluta.

Development in Marginal Outgrowths

The outgrowths from explants of the metanephros (of embryos of 8 to 10 days inclusive) were made up chiefly of four types of tissue—mesenchyme, renal epithelium, endothelium, and nervous tissue. The marginal growth began immediately after implantation. In from two to four hours after explanting, proliferation of mesenchymal cells about the margin and growth of nerve-fibers into the marginal zone could be made out. Growth of the four elements of the marginal zone increased steadily throughout the life of the culture up to 5 days. In the beginning it consisted of only a thin sheet about one cell in thickness. The most advanced edge of the outgrowing margin always remained quite thin and attenuated, but as the growth increased in width, so did it increase in depth. The different elements composing the marginal outgrowth proliferated and grew with different degrees of rapidity, but there was little difference ultimately in the extent to which they grew.

Usually the first tissue to grow was the mesenchyme; it was also the most hardy and prolific of all the tissues (Fig. 5). In appearance, these cells were typical fibroblasts connected with one another by long branching processes, which were direct extensions of the cytoplasm (Fig. 7). At first there was no definite arrangement of the mesenchyme cells; they simply grew out in a flat spreading sheet with a centripetal tendency (Figs. 4, 14). However, after 48 hours, when the growth near the explant had become thicker in different areas, definite patterns and arrangements of cells could be seen, although the attempt of the cells to assume their normal formation was often abortive and resulted in bizarre disturbances of organization (Fig. 14). This interference with the tissue in its effort to acquire its predestined form was due, no doubt, to surface tension and adhesion to the coverslip.

The renal epithelium grew out readily, but like the mesenchyme, in different forms in the various cultures. Proliferation and growth began immediately at the margin. Arising from the excretory unit (the collecting tubules, convoluted tubules, glomeruli, and undifferentiated nephrogenous tissue) growth proceeded in three fundamentally different ways: (1) with no particular formation, as in a flat sheet (Fig. 5); (2) as a perfectly organized element, carrying with it undifferentiated tissue, which later differentiated in the marginal outgrowth

(Figs. 8, 9); (3) out into the margin where an abortive attempt at formation or organization was made (Figs. 3, 4, 14). Wherever there was an end of a collecting tubule or convoluted tubule near or immediately at the margin of the culture, there was always an outgrowth of renal tissue, which appeared quite as early and grew as rapidly as the mesenchymal tissue. When growing out hand-in-hand, as it were, with other tissue of the margin, the renal tissue always appeared as a flat sheet of cubical cells closely associated with one another. This sheet was usually one cell thick and often bounded on all sides, except towards the periphery of the growth, by outgrowing mesenchymal and endothelial cells. In these flat outgrowths there was never any attempt at organization or differentiation; the outgrowth consisted simply of a multiplication of already differentiated cells.

In the event that a well-advanced marginal growth had preceded the outgrowth of renal tissue by a few hours so that there was some depth of cells into which the renal tissue could grow, then the outgrowth and differentiation occurred exactly as they did inside the explant. In other words, the collecting tubule grew into the marginal zone as a tubule intact, just as the collecting tubule grew inside the explant, carrying with it undifferentiated nephrogenic tissue which went on to complete differentiation in the margin (Figs. 8, 9, 11). The excretory tubule maintained the same relation to the collecting tubule, with regard to position and growth, that it maintained inside the explant: when it differentiated, it always did so *in situ*, *i. e.*, in the region or at the site of the angle formed by the branching of the collecting tubule, afterward becoming S-shaped by direct growth of the already differentiated cells. There was never a long, straight, direct outgrowth of the convoluted tubule such as there was in the collecting tubule.

When the marginal growth of cells was not thick, the attempts at organization were abortive, although differentiation occurred. In all marginal growths there was a certain point beyond which the entire margin grew out flat and "went wild," that is, all formation or organization was lost and the individual elements broadened out into a flat sheet. This transition could be followed in most of the cultures that survived long enough.

The third element in the marginal growth was the endothelium. As was the case with the renal tissue, the endothelium grew out in different ways. In the first place, there was a sheet-like growth, with greater intervals between the individual cells, however, than occurred in the mesenchyme or renal tissue (Fig. 3). In fixed preparations the endothelial cells were fibrillated and connected by long branching processes. No formation of blood-vascular elements was seen in this type of outgrowth. On the other hand, capillaries grew out in arciform loops from other capillaries already formed in the explant. The growth of these could be watched in detail. The development *in situ* of sinuses, blood-islands and capillaries occurred in the marginal outgrowth wherever the necessary undifferentiated tissue had been carried out from the explant. Among the tissue of the explant and marginal outgrowth, at all ages, long nerve-fibers, also ganglia, were sometimes observed. In the marginal growths, therefore, there occurred differentiation and organization of the renal parenchyma, as well as of the other basic tissue elements making up the renal body.

Differentiation, followed by a marked distortion due to the attempt of the cells to assume their usual organized positions, afforded an excellent opportunity for study. One could find places in which epithelial cells had differentiated in small flattened circular areas. Among these were highly refractive endothelial cells, intimately associated with the epithelial cells, being interwoven among them in exactly the manner noted in the development of the glomerulus. As this association of endothelium and epithelium was not observed anywhere else in the marginal growth, although there were many outgrowths of renal epithelial cells, the logical inference is that these small translucent circular areas were probably made up of cells destined to develop into the glomerulus.

DISCUSSION

Most observers agree on the growth and division of the collecting-tubule tree and the coincident splitting up of the nephrogenous tissue, thus affording a cap or covering for each branch of the collecting tubules. As far as I have been able to find, however, none of the previous observers have described the endothelial covering of these tubules, although several figures given by Herring show it.

In regard to the nephrogenic tissue, all observers agree that an S-shaped tubule is formed from a renal vesicle situated in the angle made by the collecting tubules. Ribbert (1899), was the first to call attention to the fact that a comma-shaped body preceded the formation of the tubule. Huber (1905, p. 17, Fig. 2) presents a drawing of a section of a human embryo, showing a collecting tubule, on each side of which a comma-shaped body is clearly defined. In the inferior pole of each of these bodies can be seen a renal vesicle, which Huber states differentiates out *in situ*. He does not specifically refer to the main body or the tail of the comma except to say that the renal vesicle becomes separated from the nephrogenous tissue and becomes bordered by columnar cells. The S-shaped tubule is then formed by constant growth and elongation of the renal vesicle produced by active proliferation and mitotic division of the cells. In the figure referred to above the arrangement of the cells will be found to coincide exactly with the description of my observations, the cells making up the tail and body of the comma being arranged with their axes perpendicular to the axis of the central core. Huber mentions only the sphere of cells at the lower pole and disregards

the remainder of the comma. He, as well as all other previous observers, admits that the renal vesicle develops *in situ*, but though they show in all their figures that the remainder of the comma also undergoes a definite rearrangement of cells and differentiation, still nothing but the spherical end of the nephrogenous tissue has been heretofore considered. In my observations the entire comma-shaped mass was seen to differentiate *in situ* from the undifferentiated cap of metanephric tissue and to form the S-shaped tubule.

In the formation of the convoluted tubule, from one type of tissue there are differentiated, therefore, cells of very diverse biological activity. These inherent differences are evidenced in the embryo by a difference in the growth rapidity in the several regions of the tubule anlage, and foreshadow the different functions of these respective areas in the adult.

The modus operandi of the union between the secretory and collecting tubules has never been fully described, although Schreiner hypothesizes that it occurs in given regions of the collecting tubule which are recognizable by the presence of mitotic figures. This theory is not surprising, in view of the fact that previous observations have been made on sections. In the study of the living tissue, it was possible actually to watch the uniting of the convoluted and collecting tubule, and it was seen that the convoluted tubule definitely grows into the collecting tubule.

Another very interesting fact, brought out in the cultures, was that, no matter to what extent undifferentiated tissue was displaced from the explant, it assumed within certain limits its predestined shape and activity. Nephrogenic tissue developed into secreting tubules and always became convoluted, while the reverse was true of the collecting tubules, which always grew in a comparatively straight line, regardless of the absence of pressure from surrounding structures or other physical factors that occur normally in the embryo. This, it would seem, shows that this tissue has been given a stimulus to assume a certain form and activity which will be accomplished, unless the tissue reverts, by a state of extreme activity of growth, to a spherical type of cell. This was shown in the development in the explants and also in the growth comprising the margin.

Bowman's capsule is formed from the end of the secreting tubule, which previous investigators claim invaginates to receive the coincident ingrowth of capillaries to form the glomerulus. While it might be possible to arrive at such a conclusion from an examination of sections, the study of the living tissues, either in spreads or tissue-cultures, shows that it is not the correct one. In the first place, the endothelium that differentiates *in situ* around the convoluted tubule, coincidentally with the growth and differentiation of the latter shows, from the earliest appearance of the bulbous enlargement of the glomerular end, that the wall adjacent to the overhanging first curve of the S-shaped tubule, remains intact throughout the development of the glomerulus. In the second place, in the earliest stages the dilated bulbous end of the glomerulus contains no lumen, but consists of a solid mass of spherical cells, which in appearance resemble epithelial cells. By a rearrangement of these cells there is a separation which results in the formation of a lumen within this cell mass, which leaves the epithelial cells on one side and the mass of epithelial and endothelial cells on the other.

The epithelial cells forming the peripheral wall of the tubule, however, become quite flat and very much elongated, approximating somewhat the endothelial type of cells and thus may have been mistaken for endothelial cells by other observers. According to previous writers the spherical end of the convoluted tubule increases steadily in size until the invaginating cup reaches its maximum, whereas in my observations the spherical tuft reached its maximum size before the completion of the lumen. Immediately upon completion of the lumen, the visceral and parietal layers of the anlage of Bowman's capsule are formed *in situ* within the tubule from undifferentiated spherical cells. In fixed sections, these layers of Bowman's capsule show up very clearly and it is easily seen how other investigators, considering these walls to be the boundaries of the tubule, would obtain the impression of an invaginating cup. In the fixed sections, however, interrupted stages only can be observed, whereas, in the living, the entire process of lumen development can be followed as a whole.

Concomitant with the formation of the lumen and differentiation of the cells adjacent thereto, there is differentiation of the cells within the spherical tuft, resulting in the development of endothelial cells in the most intimate association with the epithelial cells. These endothelial cells later form channel-like tracts which are covered by a single layer of epithelial cells. Hematopoiesis in the spherical tuft occurred in the cultures before any demonstrable connection with the sinuses could be seen, and it must be borne in mind that there was no circulation of blood elements whatever within the explant. At a later stage the connection of endothelial channels with the surrounding sinus was always observed to take place through the small neck of tissue connecting the spherical tuft of cells with the wall of the tubule, a communication with the ingrowing branches from the aorta being established still later.

The idea that endothelium and blood develop *in situ* was first suggested as a speculation by Herring. To him it seemed possible that the capillaries might develop *in situ* and not as ingrowths from the dorsal aorta during the formation of the glomerulus. Schreiner and Huber, on the other hand, claimed that the glomerular tuft is formed entirely by an ingrowth of pre-existing capillaries.

The later work of Sabin in blood-vessel formation, in which she states that not only the endothelium, but also the blood of the early vascular system arises *in situ*, suggests the possibility that, in other regions where primitive tissue exists, the vascular arrangement may also develop *in situ*. Certainly the observations on the development of the glomerulus in tissue cultures, where the mass of primitive mesenchyme from which this organ develops is entirely isolated from any circulation, show that not only epithelial cells, but also endothelial cells and blood-cells, differentiate *in situ* from the mass of undifferentiated spherical cells within the glomerular end of the convoluted tubule which goes to form the glomerulus. The intimate relation of the glomerular capillaries and the epithelial covering is thus explained, for it is quite difficult to figure out how this epithelial covering of each capillary could be acquired by the mere ingrowth of the capillaries into an epithelial sac. In addition to this, the sinuses also develop *in situ*, as could be seen not only from a study of spreads and tissue cultures but also in fixed sections. This development of the blood spaces *in situ* might have been surmised by Colberg (1863) from injections. He injected the blood-vessels of the kidney and found that some of the glomeruli were injected while others were not. The latter he called pseudo-glomeruli. These were undoubtedly glomeruli at an early stage of development before connection with the peripheral circulation was established. The same opinion was expressed by Herring in 1900. In 1911 Jeidell studied three injected pig embryos and found that a rich capillary network was formed in the mesenchyme surrounding the renal anlage from the inferior mesenteric and middle sacral arteries, the drainage occurring into the post-cardinal vein, inferior mesenteric veins, and capillaries of the Wolffian body. None of these injections, however, so far as I can make out from her paper and figures, penetrated farther than the primitive capsule. She concludes that the renal anlage is supplied by this means but does not state how the blood enters from the surrounding mesenchyme. In a study of human embryos of different ages, Kelly and Burnam (1914) claimed from injections that the development of the permanent blood supply of the kidney occurs when that organ reaches its definitive position and that, previous to this time, the circulation is maintained by a communication of the capillaries of the kidney with capillaries of the surrounding mesenchyme. Thus, in the ascent of the kidney, the capillaries which are left behind atrophy, while new ones form about the superior pole. At the end of seven weeks a connection with the aorta is established. Before this, no injection of the kidney could be made. Somewhat the same phenomenon occurred in the chick. Previous to complete development of the secreting tubule, I was unable to reach the sinuses or glomeruli of the metanephros by way of the peripheral circulation by injections of India ink into the heart, whereas the liver and mesonephros became so heavily injected as to appear entirely black. I think it can be concluded, therefore, that in the incompletely developed metanephros there is no continuity of or direct connection with the peripheral circulation; that there is a sinusoidal circulation, which by continued growth and constant sprouting is diminished and transformed into a capillary circulation, has been shown above. The presence of blood elements and a fluid medium inside these sinuses insures a vehicle to carry oxygen or nutritives to the developing renal body. The nourishment of the kidney in its early stages of development is therefore somewhat analogous to the nourishment of a fœtus by way of the placenta.

Sabin (1920), from studies of the blood-vascular system of living chick embryos up to four days, concludes that the various white blood-cells have different origins. The lymphocytes and monocytes arise from the endothelium lining the capillaries, while the granulocytes arise from the mesenchyme cells and migrate into the lumen of the blood-vessels. A particular study was not made of the exact origin of each type of blood-cell, but it was seen that not only the capillaries, but also the sinuses, often were filled with lymphocytes which had arisen from the endothelial walls, and in many instances, granulocytes were observed scattered among the mesenchyme cells, as though they had differentiated in that region.

Conclusions

1. In the above study of the development and growth of the metanephros of the chick embryo it is found that a continuous sheet of endothelial cells entirely surrounds the collecting and convoluted tubules.

2. The convoluted tubules differentiate *in situ* from a comma-shaped mass of undifferentiated nephrogenic tissue. The S-shaped tubule is developed out of the comma-shaped mass by different degrees of growth in different areas of the mass, and not by elongation of a renal vesicle.

3. The glomerulus, including its capillaries and blood elements, differentiates *in situ* from an undifferentiated cellular mass which completely fills the distal end of the convoluted tubule. There is no cup formation with subsequent ingrowth of capillaries.

4. The blood system surrounding the metanephric tubules is at first principally sinusoidal, as in the pronephros and mesonephros, being later almost entirely replaced by a capillary system. These sinuses are not at first in direct continuity with the capillaries of the peripheral circulation, but circulation takes place by diffusion between them and the capillaries.

5. The convoluted tubules grow directly into the intact collecting tubules.

6. In tissue cultures of the metanephros there does not occur a dedifferentiation of tissue but an active growth and differentiation takes place not only in the explant but also, within certain limits, in the marginal outgrowth.

BIBLIOGRAPHY

Atterbury, R. R., 1920. Potentialities of the different parts in the kidney anlage. Anat. Rec., vol. 18-19, p. 219.

Carrel and Burrows, 1910. Cultivation of adult tissues and organs outside the body. Jour. Amer. Med. Assn., vol. 5.

Champy, C., 1913-14. Quelques resultats de la methode de culture des tissues; généralités. (Note préliminaire.) Arch. de zool. expér. et gén., t. 3, pp. 42-51.

Idem, 1914. Quelques resultats de la methode des cultures de tissus; le rein. Arch. de zool. expér. et gén., t. 4, pp. 307-386.

Colberg, A., 1863. Zur Anatomie der Niere. Centralbl. med. Wissensch., Bd. 1.

Disse, J., 1902. Harn und Geschlectsorgane. Von Bardelebens Handbuch der Anatomie des Menschen, Bd. 7, Jena.

Felix, W., 1912. The development of the urinogenital organs. Human Embryology, Keibel and Mall, vol. 2.

Herring, P. T., 1900. The development of the Malpighian bodies of the kidney and its relation to pathological changes which occur in them. J. Path. and Bact., vol. 6.

Huber, C. G., 1905. On the development and shape of uriniferous tubules of certain of the higher mammals. Amer. J. Anat., vol. 4.

Jeidell, H., 1911. A note on the source and the character of the early blood vessels of the kidney. Anat. Rec., vol. 5.

Kelly, H. A., and C. Burnam, 1914. Diseases of Kidney Ureters and Bladder: Development of the renal vascularization.

Lillie, F. R., 1919. The Development of the Chick.

Minot, C. S., 1910. A Laboratory Text-book of Embryology.

Minoura, T., 1921. A study of testis and ovary grafts on the hen's egg and their effects on the embryo. J. Exper. Zool., vol. 33.

Ribbert, H., 1899. Ueber die Entwicklung der bleibenden Niere und über die Enstehung der Cystenniere. Verhandl. der deutsch. path. Gesellsch., p. 18.

Sabin, F. R., 1920. Studies on the origin of the blood-vessels and of the red blood-corpuscles as seen in the living blastoderm of chicks during the second day of incubation. Contributions to Embryology, vol. 9; Carnegie Inst. Wash., Pub. 272.

Schreiner, K. E., 1902. Ueber die Entwicklung der Amniotenniere. Zeitschr. f. wiss. Zool., Bd. 71.

Stoerk, O., 1904. Beitrag zur Kenntnis des Aufbaus der menschlichen Niere. Anat. Hefte, Bd. 1, 23.

EXPLANATION OF FIGURES

Plate 1.

Fig. 1.—16 mm. lens. Seven days' growth of metanephros of 8-day chick embryo. Extensive growth of the collecting-tubule tree not only within the explant but also out into the marginal growth. The collecting tubules have grown into the margin as intact tubules, branching in the characteristic dichotomous manner, the line of growth being a relatively straight one. In this figure the branches are all intralobar, the main stem to the left of the figure being a quarternary branch. The large clear areas between the basic branches are the sinuses; smaller ones are seen toward the periphery. Throughout the culture there are several displaced collections of undifferentiated nephrogenous tissue. The margin of the growth appears in the lower right hand corner of the figure.

Fig. 2.—16 mm. lens. Seven days' growth of the metanephros of a 10-day chick embryo. Entire excretory unit completely differentiated inside the explant. Union between the convoluted and collecting tubules has occurred in most instances. The main stem of the collecting tubule here is made up of two tertiary tubules. In the upper portion of the figure there are some convoluted tubules which have grown more nearly in a straight line than usual. All of the convolutions show typical dilation of the glomerular ends. The glomeruli here are quite small. Numerous macrophages can be seen throughout the culture clearing up the remaining undifferentiated tissue by phagocytosis.

Fig. 3.—16 mm. lens. Seven days' growth of metanephros of 8-day chick embryo. Marginal outgrowth of collecting tubules on the right. The tubules grew out intact within the thicker region but became flattened out into a sheet-like growth in the more attenuated portion of the margin. At different points in the marginal outgrowth can be seen endothelium composed of cells larger and flatter than the mesenchyme cells, which make up the greater part of the outgrowth. Owing to the heavy staining, the structures inside the explant cannot be made out. Formed sinuses in the explant at the left are well shown about the base of the collecting-tubule outgrowth.

Plate 2.

Fig. 4.—16 mm. lens. Six days' growth of metanephros of 8-day embryo. Extensive outgrowth of formed collecting tubule into margin, flattening in the attenuated edge of the culture.

Fig. 5.—16 mm. lens. Seven days' growth of metanephros of 8-day embryo. Extensive outgrowth of renal epithelium from collecting tubule in the explant. There is a lumen in this for a short distance in the marginal growth. The endothelium can be seen about the tubule and is quite characteristic in this mode of outgrowth. This endothelial growth originates from the endothelial covering about the tubules and presents the same type of outgrowth that occurs from endothelium elsewhere.

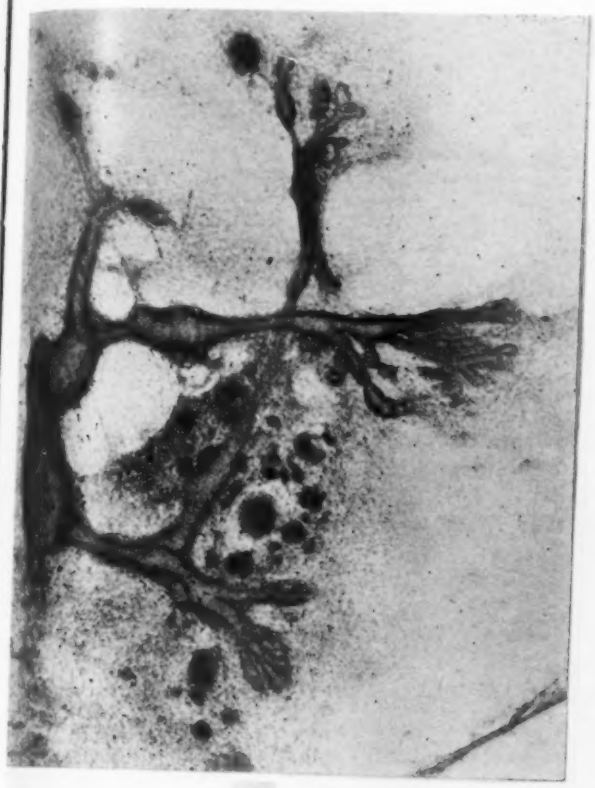
Fig. 6.—4 mm. lens. Four days' growth of metanephros of 9-day chick embryo. Iodine vapor. Comparatively straight collecting tubule into which the convoluted tubule has grown. The point of union is shown in the lower left hand corner of the figure, where the convoluted tubule appears to be constricted. The tubules are sharply defined by the characteristic etched-like endothelium. The lumen formation in the glomerulus has been completed and the capsule of Bowman is well shown. The tuft of epithelial-like cells, among which endothelial are intermingled, shows as yet no evidence of vascularization. This figure demonstrates clearly the ease with which the terminal walls of the convoluted tubule can be seen and also how these intratubular changes can be distinguished from those taking place outside the tubule. About the tubules many white blood-cells and phagocytes are evident. Vacuolization of cells, exactly as occurred in the formation of the sinuses, is well shown in the lower right quadrant of the figure.

Fig. 7.—Seven days' growth of metanephros of 8-day chick embryo, illustrating the same points as figure 5.

Plate 3.

Fig. 8.—Five days' growth of metanephros of 9-day chick embryo; 16 mm. lens. Collecting tubules have grown straight out into the marginal growth, carrying with them the undifferentiated nephrogenic tissue which went on to complete differentiation in their final position. They have grown out as intact tubules. The characteristic type of growth for the collecting and convoluted tubules is well shown, the former growing in a straight line and the latter being coiled. At the uppermost point in the figure there is a very small glomerulus in which red blood-cells can be seen. Union of the convoluted and collecting tubules has occurred.

Fig. 9.—Higher power picture of Figure 8; 4 mm. lens, showing extreme upper pole of collecting tubule, convoluted tubule, and glomerulus. The characteristic convolutions of the secreting tubule are well shown, despite the fact that the growth is in the margin where the physical factors attending it were entirely different from those obtaining in the embryo. The glomerulus



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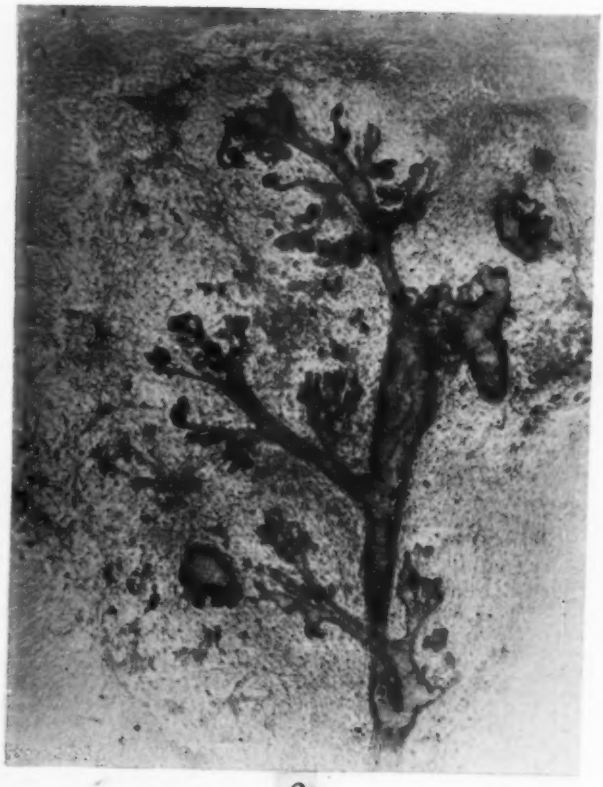

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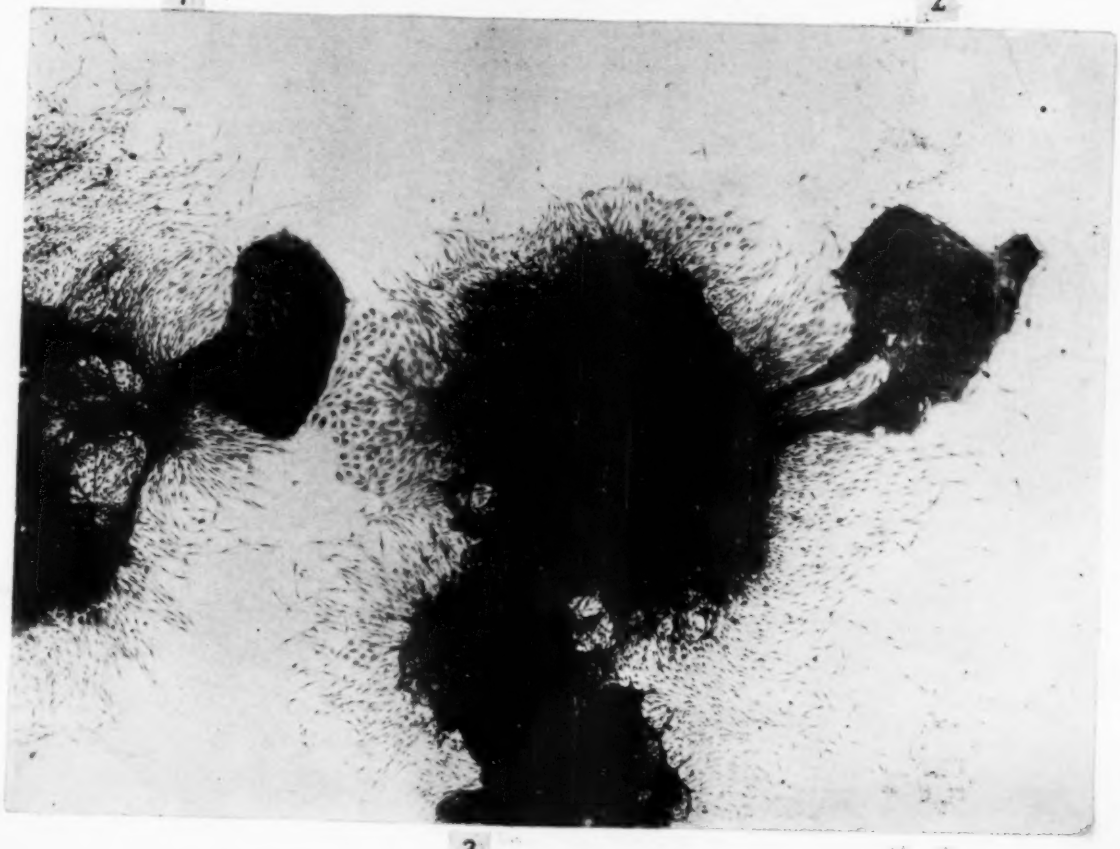

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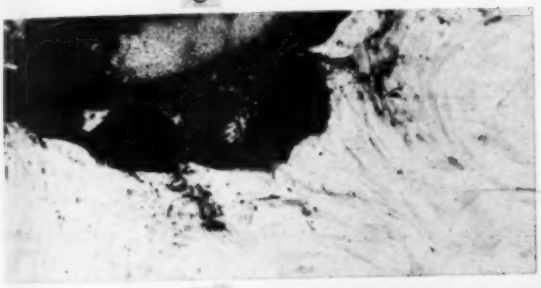

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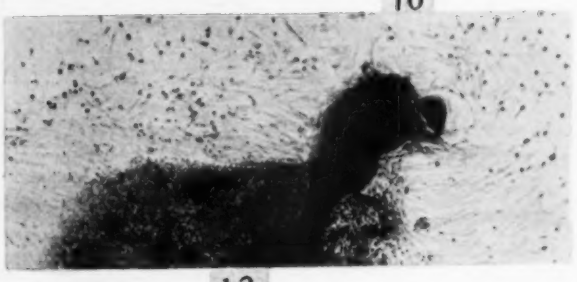

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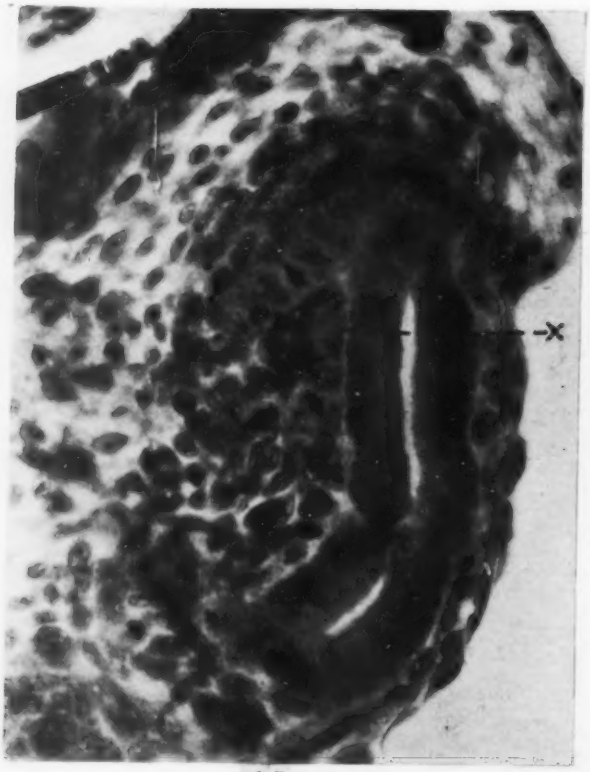

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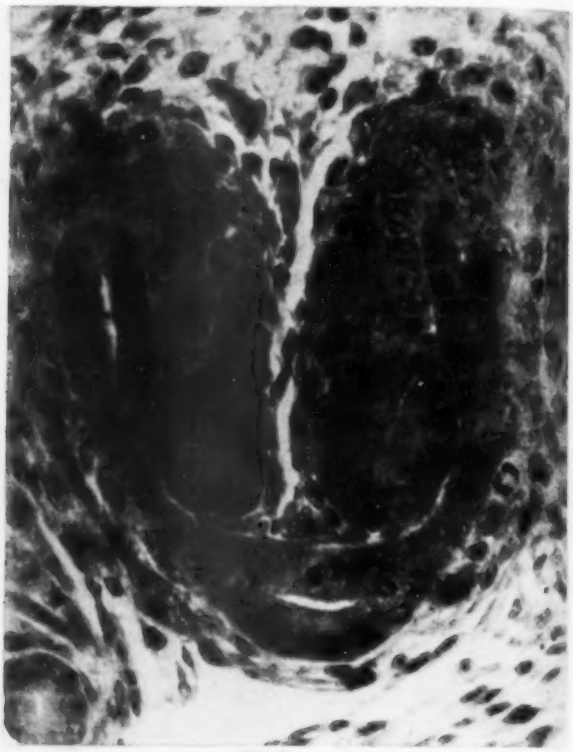

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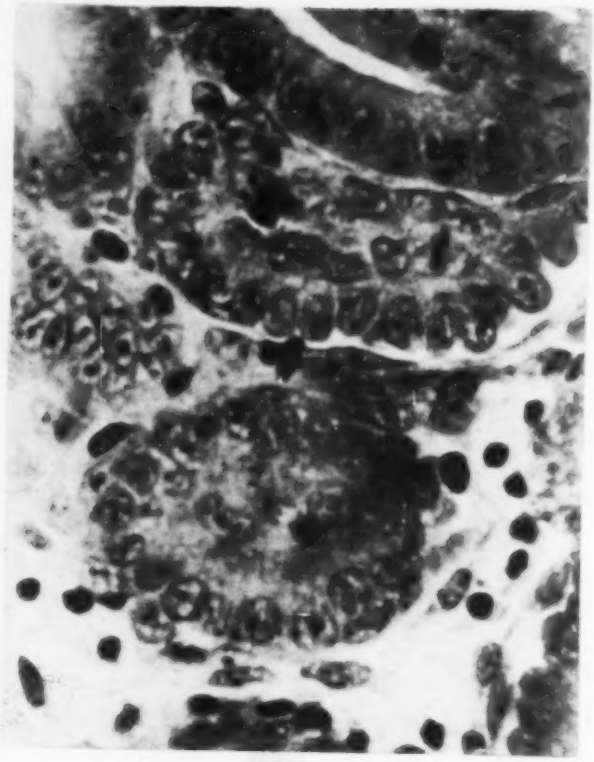

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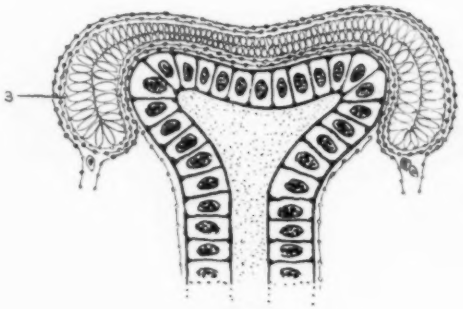

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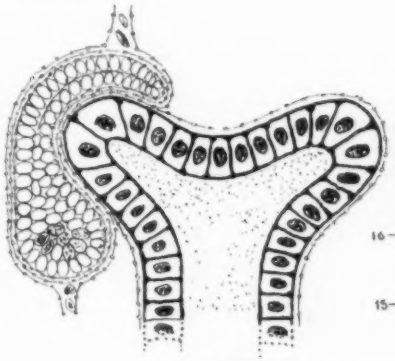

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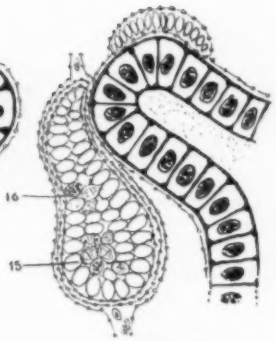

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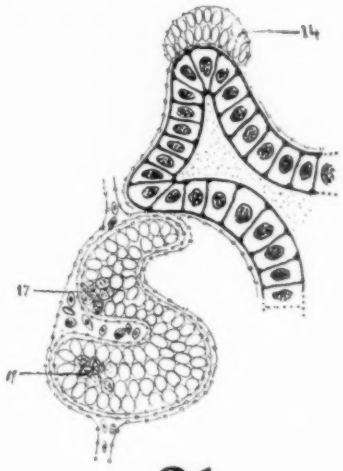

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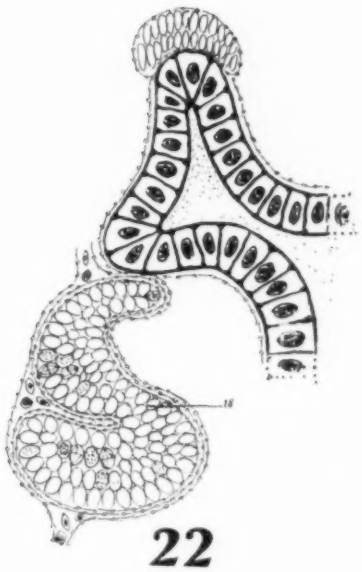

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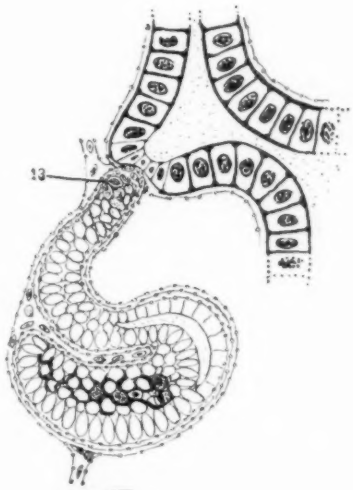

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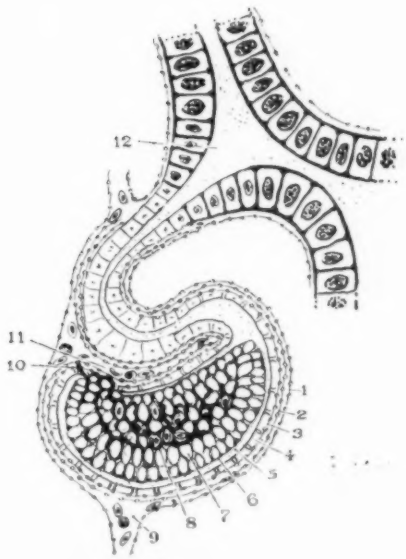

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appears as a small sphere in which red blood-cells are seen as black dots. The defining endothelial cells surrounding the tubules show plainly.

FIG. 10.—4 mm. lens. Three days' growth of metanephros of 9-day chick embryo. Small explant containing collecting tubule, which presents a very interesting growth. The forward or normal growth is accompanied by the usual dichotomous type of branching, while the posterior growth is in a straight line, with no tendency to branch. On either side of the collecting tubule, in the angles, there are two S-shaped tubules which differentiated in their characteristic position as regards the collecting tubule. These S-shaped tubules differentiated in the culture. The right side is at a more advanced stage than the left side. Certainly the tendency of the convoluted tubules to be coiled in this culture may be attributed to an inherent tendency of the cells. The collecting tubule, for the same reason, has grown in a straight line.

FIG. 11.—4 mm. lens. Four days' growth of metanephros of 9-day chick embryo. Immediately at the beginning of the marginal outgrowth there are two convoluted tubules with their glomeruli in the course of development. The one to the right side is not as far advanced as the one to the left. Lumen formation has occurred in the tubule to the left. The tubule to the right is a solid core of cells with a dilated bulbous end in which the formation of a lumen has not as yet occurred. The characteristic pinched-off appearance of the neck of the glomerulus, which results from the relatively slow growth of the cells composing this region, is shown.

FIGS. 12 and 13.—Five days' growth of metanephros of 9-day chick embryo. Sinus formation well shown about the convoluted tubules. The white blood-cells show up as dark spots, more pronounced in Figure 12.

FIG. 14.—Same as Figures 12 and 13, except that the sinuses are located more in the denser region about the convoluted tubules.

PLATE 4.

FIG. 15.—Metanephros, 8-day embryo. Section 5 micra. × 750. Collecting tubule formation with inverted comma-shaped mass of nephrogenic tissue about end. The cells making up the body and tail of comma are spherical in type with their axes perpendicular to the central core. In the head of the comma there is marked proliferation evidenced by mitotic figures. About the periphery of the comma-shaped mass the endothelial cells can be seen with their axes parallel to the central core. These endothelial cells are just in a stage of transformation from the round spherical type to the long slender endothelial type. The head of the comma is turned somewhat towards the observer and at the inner angle about the middle of the mass (at X) is an endothelium-lined space which approximates the sphere and, when several sections are studied, proves to communicate with an endothelium-lined sinus, which is somewhat poorly shown in this figure, just beneath the head of the comma. This is the portion of the sinus surrounding the comma-shaped mass that will later be included in the first curve of the S-shaped tubule. This figure shows the comma just prior to the development of the lumen and demonstrates very clearly that the tail and body of the comma, as well as the head, are utilized in the formation of the S-shaped tubule.

FIG. 16.—Metanephros, 9-day embryo. Section 5 micra. × 750. A "Y"-shaped collecting tubule with two comma-shaped bodies along its inner sides and about its ends. Between the commas there is an endothelium-lined sinus which surrounds the entire anlage. The endothelial walls of the sinus are in opposition along the lateral side of each of the collecting tubules, but the connection with the large sinus at the lower portion of the figure can be traced even in the photograph. The tubule is here shown in a later stage of development than in Fig. 1. The endothelial covering is evident, as well as the endothelium lining the sinus, the two layers being in apposition. Lumen formation has just begun and is somewhat more advanced on the right side than on the left. The characteristic polarity of the endothelial and epithelial cells, as well as the more rapid proliferation of the spherical head pole of the tubule, is well shown.

FIG. 17.—Metanephros, 9-day embryo. Section 5 micra. × 1500. Cellular tuft in glomerular end of convoluted tubule shown immediately below a loop of the tubule. The lower loop of tubule belongs to another unit. The capsule of Bowman is partially formed about the spherical tuft of cells and the tuft is bounded by a row of radially arranged epithelial-like cells. The flattened epithelial cells making up the parietal layer of Bowman's capsule are evident. The point of junction of the glomerular end with the convoluted tubule occurs just to the right in the figure. A double line of flattened endothelial cells running across the superior surface of the glomerular tuft is evident. In the distal end of the tuft a black spot is seen which is a newly formed red blood-cell. On each side of this cell there are endothelial cells which form a channel in the middle of the epithelial cells making up the major portion of the tuft. This is better shown in Plate 5. A mitotic figure can be seen towards the proximal end of the tuft. The clear areas in the figure, in which red blood-cells appear as black spots, are the sinuses in the immediate neighborhood of the glomerulus.

PLATE 5.

FIG. 18.—Collecting tubule covered by cap of metanephrogenic tissue. The collecting tubule contains granules and is lined with columnar epithelium. Three layers of endothelium can be seen about the cap and one layer surrounding the collecting tubule. The spherical epithelial cells composing the cap are radially arranged about a central core corresponding to the single line shown. Blood cells are shown in the sinus existing between the two outermost endothelial layers.

FIG. 19.—Collecting tubule buds increased in length. The metanephrogenic cap has become transformed into an inverted comma-shaped mass. The cells making up the tail of the comma show the same arrangement as in Fig. 18. The head of the comma is the seat of most marked proliferation causing a dilatation of the head pole.

FIG. 20.—Collecting tubule only partly shown. Growth of bud extending peripheralward carrying small portion of metanephrogenic tissue. The comma-shaped mass has approximated its definitive position in regard to the collecting tubule. At points 15 and 16 the most rapid proliferation of cells takes place.

FIG. 21.—Transition of comma-shaped mass into that of an S-shaped tubule has occurred, angulation having been brought about by proliferation and more rapid growth of cells at points 17 and 19, point 17 being the first curve of the S-shaped tubule formed. A sac-like protrusion of the sinus (No. 11 in Fig. 24) has been included between the superior surface of the bulbous glomerular end (19) and the inferior surface of the first curve (17). Blood cells are shown in this sac. Metanephrogenic cap shown at 14.

FIG. 22.—Collecting tubule and metanephrogenic cap remain about the same as in Fig. 21. S-shaped tubule more angulated. Second curve No. 18. First curve increased in size. Glomerular end a dilated bulb of spherical epithelial cells.

FIG. 23.—S-shaped tubule growing in the collecting tubule at point 13. Lumen formation occurring in second curve with abutting cells becoming cuboidal in shape. Heavy black lines in tuft of epithelial cells composing glomerular end are the endothelial cells, No. 8 in Fig. 24.

FIG. 24.—S-shaped tubule completely joined with collecting tubule. Lumen complete with the formation of Bowman's capsule (5). Note that this formation is entirely inside the endothelial covering layers 1, 2 and 3, 4 constituting the parietal layer of Bowman's capsule and 6 the visceral; 7, spherical epithelial cells composing the tuft surrounded by Bowman's capsule. Intertwining endothelial cells and channels (8) among the epithelial cells. Blood elements are also shown forming *in situ*. The connection of the endothelium inside the tuft with the surrounding sinus is shown at 10, and the included endothelial sac or sinus at 11; 9, a connection between the surrounding endothelial sinus and sinuses in other parts of the metanephros. The lumen of the collecting tubule is shown at 12.

BIOMETRICAL STUDIES IN PATHOLOGY. II

PATHOMETRIC INDEX NUMBERS:

PRELIMINARY ACCOUNT OF A QUANTITATIVE METHOD OF EVALUATING PATHOLOGICAL PHENOMENA[1]

By RAYMOND PEARL[2]

THE PROBLEM

An autopsy protocol is essentially and in the main a qualitative description of the state of the organs of the body at death. Its fundamental purpose is to make possible in some degree the reconstruction of the course of physiological (or pathological) events which led to death, so that a correlation may be effected between clinical findings and the actual state of the organs of the body. That this is a most important and useful purpose, which in proportion as it is fulfilled aids enormously the progress of scientific medicine, no thoughtful person will deny.

But it is possible to conceive a still broader and more fundamental usefulness of pathological anatomy even than this which has been sketched, namely, as an aid in the solution of problems of general biology. Pathological phenomena are biological phenomena. The decay and breakdown of organs is just as much and just as truly an essential part of the whole normal life history of an organism as are, for example, growth phenomena. That pathological anatomy is not usually thought of by biologists as an intrinsic part of the subject matter of their science is really only the expression of an accident of specialization in human activity. But because of the fact that, in the main, pathology has been developed primarily for its strictly medical usefulness, it has resulted that its broader biological aspects have been, if not neglected, at least not developed to the extent that seems possible. There are almost innumerable biological problems presented by the data of pathology which have not been touched even. A certain restricted group of these problems has interested me greatly, and the present paper is offered as a first attempt, subject to elaboration and revision with further study, at an attack upon them.

If one reads over carefully a great number of autopsy protocols something like the following thought forces itself upon his mind. There is a great deal of variation in the amount and extent of observable pathological lesions associated with death. In some cases one sees enormous alterations of structure and function affecting nearly or quite all the important organs of the body. In other cases all that can be found at autopsy are relatively slight, or even apparently insignificant lesions of a few organs. Between these two extremes are all degrees of variation in the pathological picture. The whole situation, looked at in a naïve way, is as though it required a great deal more disturbance of structure and function to kill some people than it does to kill others. In other words, while the whole pathological picture, as presented in the protocol, is essentially a qualitative one, nevertheless as soon as one starts to think about it, he wants to think in quantitative terms.

Such thinking at once leads to some further considerations. There is suggested a concept, which for lack of a better term, may be called that of the *specific vital resistance* of an individual. If individual *A* lives along for a considerable period of time with a number of extremely grave organ lesions, while individual *B* dies promptly upon the appearance of a few relatively slight disturbances of structure and function, we may conceive that *A* has a higher specific vital resistance to death than *B*. It takes more to kill *A* than it does *B*. If we can get some sort of reliable quantitative measure of what we have called specific vital resistance it will at once open out many lines of research. How do the different races and the two sexes, for example, compare in respect of this presumably fundamental biological quality?

The converse of the concept of specific vital resistance is that of *specific pathopoiesis*,[3] the tendency of the individual to become pathological, or to develop alterations

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[2] Assisted by Agnes Latimer Bacon.

[3] The use of this term quite obviously puts much weight upon the part played by the individual, *qua* individual, in his own becoming diseased. This is deliberate, and in accord with the writer's general view, that biologically the innate constitution of the host organism is at least as important a factor, broadly speaking, in the etiology of disease, as is the invading organism, even in most infectious diseases, and probably a much more important factor than any or all external agents in the etiology of the so-called "organic" diseases.

of structure and function from the normal. According to this concept the individual, who, upon autopsy, exhibits many and grave lesions is more highly pathopoietic than another who shows only slight and insignificant lesions. Is the negro, in this sense, more highly pathopoietic than the white?

There are many angles of approach and possibilities inherent in this general idea which has been sketched. Will a proper quantitative measure of the general autopsy picture tend to have higher or lower values in those dying, for example, primarily because of disturbances in the functioning of the heart, than in those dying primarily because of disturbances in the functioning of the liver? And similarly for other organs.

I shall not attempt in this preliminary paper to outline the problems which can be attacked if a proper method of quantitatively evaluating a general pathological picture can be devised. Instead I shall leave this to the imagination and insight of the reader, and proceed to state the method by which I have approached the problem of setting up such a measure.

A Suggested Pathometric Index Number

In developing a pathometric index number I have followed the same idea that was developed in my laboratory [Pearl and Surface (1909), and Pearl (1912)] some years ago in a series of papers, in connection with the study of certain problems of genetics. It was there pointed out that:

"An 'index number' in the sense here used means a single mathematical function which combines in itself the values of several independent or correlated variables. In such a function each of the variables may be weighted in any desired manner to meet the needs of the particular problem. Having decided the relative degree to which each variable shall be weighted, the index number as finally calculated gives an absolutely impartial and impersonal summing up of the total combined value or effect of the variables entering into it.

"The theory of a breeding index number may be illustrated by a concrete example. Let the matter of improving maize by selective breeding be taken. Suppose that a breeder starts with a promising variety of yellow dent corn. This variety, while promising, has never been improved by breeding at all. The ears are only fair in respect to size and shape. The principal aim of the breeder of this corn, let us suppose, is to increase the 'earliness' (*i. e.*, shorten the time of maturing), but at the same time he wishes to improve the other characteristics of the corn—size and shape of ear, relative proportion of corn on cob, and yield per acre. In making field selections of plants to furnish seed for testing out by the 'ear-row' or other method, the breeder will endeavor to select with reference to as many as possible of the points enumerated above in addition to 'earliness.' Further it will be desired after the ears have been harvested and dried to take careful account in selecting the seed of at least the following points: Shape of ear, length of ear, circumference of ear, condition of tip and butt of the ear, kernel shape, germination and proportion of shelled corn to cob. Now if a plant happens to be specially early, even though it be relatively poor in respect to these ear characters, it is likely to be selected to furnish seed, in spite of these defects. But it is possible to devise a formula for a selection index number which shall give whatever weight may be previously agreed upon to each of these several variable characters of the corn which have been enumerated. Having settled upon a particular formula, the selection of seed then becomes essentially a purely mechanical matter so far as the characters included in the formula are concerned. The value of the index will be determined by the relative contributions from each of the included variables. If the breeder calculates such a selection index number, and takes no ears with an index below that standard, it will then be possible for him to select with reference to a series of characters in an unbiased and impartial manner."

The quantification of the whole pathological picture presented by an autopsy protocol offers a closely similar problem to that dealt with in forming breeding index numbers.

There are certainly four elements as factors in the case which must be considered in making up a pathometric index number. There are:

1. *The magnitude and gravity of the lesion, as lesion.*—A single small apical scar in the lung is a slight and insignificant lesion of tuberculosis, as compared with a large ulcerating cavity resulting from the presence in the lungs of tubercle bacilli. The comparative extent and gravity of any given lesion, as compared with other lesions of the same sort, may be indicated by a numerical weighting on a scale of 1 to 10, 1 denoting the smallest and most significant lesion of the sort dealt with, and 10 the largest and most grave ever found. The assignment of the weights will depend, of course, upon the careful study of the description in the protocol, and upon the judgment and knowledge of the worker. If the *same* individual does all the weighting, however, on a given series of autopsies, the results will be comparable *inter se*.

2. *The organ or organ system in which the lesion occurs.*—It is obvious *a priori* that any lesion, however insignificant or grave, is more important pathologically, *i. e.*, to life or death, if it occurs in some organs than if it occurs in some others, because some organs are fundamentally more important to the general physiological economy than others. Thus, for example, a lesion of the skin, say an epithelioma, is much less likely *per se* to cause death, than a carcinoma of the liver. How shall this idea be expressed quantitatively? In general terms the answer is obvious; we should give numerical weights to the several organ systems in proportion to their importance in the general physiological economy. After careful thought about the matter, it has seemed that the best *available* indication of the relative importance of the several organ systems, is to be found in the relative frequency with which their breakdown (structural and functional) leads to death. In an earlier study (Pearl, 1920) I have allocated the death rates from the several statistically recognized causes of death back to the organ systems, the breakdown of which is primarily responsible for the lethal effect of that cause. As a first approximation to organ weightings I have taken from that paper (which should be read in connection with the present study) the following death-rates as weighting figures.

Organ System	*Weight*
Respiratory system	396
Alimentary canal, and associated organs of metabolism	335
Circulatory system, blood and blood forming organs	210
Nervous system and sense organs	176
Kidneys and related excretory organs	107
Primary and secondary sex organs	88
Skeletal and muscular system	13
Skin	10
Endocrinal system	2
"Body as a whole"	1507

These weights represent the proportion in which breakdown of the indicated organ systems actually do cause death in the United States Registration Area, so far as may be judged from certified causes of death.

The rubric "Body as a whole" with a weight which is the sum of the separate organ weights, is included to care for pathological conditions not referable, on the basis of any present knowledge, to any particular organ system, to the exclusion of others but which involve the whole body as a functioning machine. Such cases are mainly profound disturbances of metabolism, such as, for example, rickets.

3. *The etiological factor involved in the production of the lesion.*—The influences upon the chances of life or death of the individual vary greatly, with lesions of the same extent in the same organ, according to the etiological factor behind the lesion. For example, a traumatic lesion of the skin, as from a burn, is a very different thing in respect of prognosis than an equally extensive lupus lesion. As a first attempt at the quantification of this idea the following system of etiological weights has been set up:

Etiological Factor	*Weight*
Malignant neoplasm	10
Infection	8
Poison (inorganic or organic)	6
Trophic or organic functional disturbance	5
Trauma	3
Structural (inherited or congenital) abnormality	2
Benign neoplasm	1
Non-bacterial parasites (e.g. worms)	1

The assignment of these weights is entirely arbitrary but tries in a general way to base itself upon the prognosis in the presence of these various etiological factors.

4. *The age of the person.* A lesion of given magnitude, in a particular organ system, and of a given etiology, has a different pathological significance if present in a baby, to what it has if present in a man of 70 years, let us say. In general, the age of the individual must surely have a place in any quantification of the pathological picture. In the pathometric index about to be described it has been decided, after trial of several other plans, to introduce the age factor in this form of the actuarial constant $\mathring{e}_x$, which is the complete expectation of life at a given age.

How now shall these four essential elements in the problem be combined to form a single index number? After careful consideration and experimentation the following function has been chosen.

$$I_p = \frac{\Sigma \left\{ \left(\sqrt[5]{b^a} \right) \right\} c}{1000} \times \mathring{e}_x$$

In this expression

I_p = pathometric index number for a particular autopsy protocol.
Σ = summation to include each and all lesions present in the case.
b = organ system weight.
a = lesion weight.
c = etiological weight.
$\mathring{e}_x$ = complete expectation of life at attained age of individual, from life table.

The function

$$\left(\sqrt[5]{b^a} \right) c$$

can obviously be tabled once for all for the 10 assigned weights of b, and values of a and c ranging from 1 to 10 inclusive. Such tables have been calculated and are here given as Tables 1 to 10.

TABLES OF FUNCTION $\left(\sqrt[5]{b^a} \right) c$ FOR ASSIGNED VALUES OF b.

TABLE 1. $b = 2$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	1.15	1.32	1.52	1.74	2.00	2.30	2.64	3.03	3.48	4.00
2	2.30	2.64	3.03	3.48	4.00	4.59	5.28	6.06	6.96	8.00
3	3.45	3.96	4.55	5.22	6.00	6.89	7.92	9.09	10.45	12.00
4	4.59	5.28	6.06	6.96	8.00	9.19	10.56	12.13	13.93	16.00
5	5.74	6.60	7.58	8.71	10.00	11.49	13.19	15.16	17.41	20.00
6	6.89	7.92	9.09	10.45	12.00	13.78	15.83	18.19	20.89	24.00
7	8.04	9.24	10.61	12.19	14.00	16.08	18.47	21.22	24.38	28.00
8	9.19	10.56	12.13	13.93	16.00	18.38	21.11	24.25	27.86	32.00
9	10.34	11.88	13.64	15.67	18.00	20.68	23.75	27.28	31.34	36.00
10	11.49	13.19	15.16	17.41	20.00	22.97	26.39	30.31	34.82	40.00

TABLE 2. $b = 10$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	1.58	2.51	3.98	6.31	10.00	15.85	25.12	39.81	63.10	100.00
2	3.17	5.02	7.96	12.62	20.00	31.70	50.24	79.62	126.19	200.00
3	4.75	7.54	11.94	18.93	30.00	47.55	75.36	119.43	189.29	300.00
4	6.34	10.05	15.92	25.24	40.00	63.40	100.48	159.24	252.38	400.00
5	7.92	12.56	19.91	31.55	50.00	79.24	125.59	199.05	315.48	500.00
6	9.51	15.07	23.89	37.86	60.00	95.09	150.71	238.86	378.57	600.00
7	11.09	17.58	27.87	44.17	70.00	110.94	175.83	278.68	441.67	700.00
8	12.68	20.10	31.85	50.48	80.00	126.79	200.95	318.49	504.77	800.00
9	14.26	22.61	35.83	56.79	90.00	142.64	226.07	358.30	567.86	900.00
10	15.85	25.12	39.81	63.10	100.00	158.49	251.19	398.11	630.96	1000.00

TABLE 3. $b = 13$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	1.67	2.79	4.66	7.78	13.00	21.71	36.27	60.58	101.18	169.00
2	3.34	5.58	9.32	15.57	26.00	43.43	72.54	121.15	202.36	338.00
3	5.01	8.37	13.98	23.35	39.00	65.14	108.80	181.73	303.54	507.00
4	6.68	11.16	18.64	31.13	52.00	86.85	145.07	242.31	404.72	676.00
5	8.35	13.95	23.30	38.92	65.00	108.57	181.34	302.89	505.90	845.00
6	10.02	16.74	27.96	46.70	78.00	130.28	217.61	363.46	607.08	1014.00
7	11.69	19.53	32.62	54.48	91.00	152.00	253.87	424.04	708.26	1183.00
8	13.36	22.32	37.28	62.27	104.00	173.71	290.14	484.62	809.45	1352.00
9	15.03	25.11	41.94	70.05	117.00	195.42	326.41	545.19	910.63	1521.00
10	16.70	27.90	46.60	77.83	130.00	217.14	362.68	605.77	1011.81	1690.00

TABLE 4. $b = 88$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	2.45	6.00	14.68	35.94	88.00	215.47	527.56	1291.73	3162.78	7744.00
2	4.90	11.99	29.36	71.88	176.00	430.93	1055.13	2583.46	6325.56	15488.00
3	7.35	17.99	44.03	107.82	264.00	646.40	1582.69	3875.19	9488.34	23232.00
4	9.79	23.98	58.71	143.76	352.00	861.86	2110.26	5166.93	12651.12	30976.00
5	12.24	29.98	73.39	179.70	440.00	1077.33	2637.82	6458.66	15813.90	38720.00
6	14.69	35.97	88.07	215.64	528.00	1292.80	3165.39	7750.39	18976.68	46464.00
7	17.14	41.97	102.75	251.58	616.00	1508.26	3692.95	9042.12	22139.46	54208.00
8	19.59	47.96	117.42	287.53	704.00	1723.73	4220.52	10333.85	25302.23	61952.00
9	22.04	53.96	132.10	323.47	792.00	1939.20	4748.08	11625.58	28465.01	69696.00
10	24.48	59.95	146.78	359.41	880.00	2154.66	5275.65	12917.32	31627.79	77440.00

TABLE 5. $b = 107$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	2.55	6.48	16.51	42.02	107.00	272.43	693.65	1766.09	4496.07	11449.00
2	5.09	12.97	33.01	84.05	214.00	544.87	1387.29	3532.19	8993.34	22898.00
3	7.64	19.45	49.52	126.07	321.00	817.30	2080.94	5298.29	13490.00	34347.00
4	10.18	25.93	66.02	168.10	428.00	1089.73	2774.58	7064.38	17986.67	45796.00
5	12.73	32.41	82.53	210.12	535.00	1362.17	3468.23	8830.47	22483.34	57245.00
6	15.28	38.90	99.03	252.15	642.00	1634.60	4161.87	10596.57	26980.01	68694.00
7	17.82	45.38	115.54	294.17	749.00	1907.03	4855.52	12362.67	31476.68	80143.00
8	20.37	51.86	132.04	336.20	856.00	2179.47	5549.16	14128.76	35973.34	91592.00
9	22.91	58.34	148.55	378.22	963.00	2451.90	6242.81	15894.85	40470.01	103041.00
10	25.46	64.83	165.06	420.25	1070.00	2724.34	6936.45	17660.95	44966.68	114490.00

TABLE 6. $b = 176$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	2.81	7.91	22.25	62.58	176.00	495.01	1392.22	3915.80	11013.44	30976.00
2	5.63	15.82	44.50	125.15	352.00	990.02	2784.44	7831.60	22026.87	61952.00
3	8.44	23.73	66.75	187.73	528.00	1485.03	4176.66	11747.40	33040.31	92928.00
4	11.25	31.64	89.00	250.31	704.00	1980.05	5568.88	15663.19	44053.75	123904.00
5	14.06	39.55	111.24	312.88	880.00	2475.06	6961.10	19578.99	55067.19	154880.00
6	16.88	47.46	133.49	375.46	1056.00	2970.07	8353.32	23494.79	66080.62	185856.00
7	19.69	55.37	155.74	438.03	1232.00	3465.08	9745.54	27410.59	77094.06	216832.00
8	22.50	63.28	177.99	500.61	1408.00	3960.09	11137.76	31326.39	88107.50	247808.00
9	25.31	71.19	200.24	563.19	1584.00	4455.10	12529.98	35242.19	99120.93	278784.00
10	28.13	79.11	222.49	625.76	1760.00	4950.11	13922.20	39157.98	110134.37	309760.00

TABLE 7. $b = 210$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	2.91	8.49	24.74	72.07	210.00	611.88	1782.82	5194.59	15135.43	44100.00
2	5.83	16.98	49.47	144.15	420.00	1223.75	3565.64	10389.17	30270.86	88200.00
3	8.74	25.47	74.21	216.22	630.00	1835.63	5348.45	15583.76	45406.29	132300.00
4	11.65	33.96	98.94	288.29	840.00	2447.50	7131.27	20778.34	60541.72	176400.00
5	14.57	42.45	123.68	360.37	1050.00	3059.38	8914.09	25972.93	75677.15	220500.00
6	17.48	50.94	148.42	432.44	1260.00	3671.25	10696.91	31167.51	90812.58	264600.00
7	20.40	59.43	173.15	504.51	1470.00	4283.13	12479.73	36362.10	105948.01	308700.00
8	23.31	67.92	197.89	576.59	1680.00	4895.00	14262.54	41556.68	121083.44	352800.00
9	26.22	76.41	222.63	648.66	1890.00	5506.88	16045.36	46751.27	136218.87	396900.00
10	29.14	84.90	247.36	720.73	2100.00	6118.76	17828.18	51945.85	151354.30	441000.00

TABLE 8. $b = 335$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	3.20	10.23	32.74	104.72	335.00	1071.65	3428.17	10966.59	35081.70	112225.00
2	6.40	20.47	65.47	209.44	670.00	2143.30	6856.35	21933.19	70163.40	224450.00
3	9.60	30.70	98.21	314.16	1005.00	3214.96	10284.52	32899.78	105245.11	336675.00
4	12.80	40.93	130.94	418.89	1340.00	4286.61	13712.69	43866.37	140326.81	448900.00
5	15.99	51.17	163.61	523.61	1675.00	5358.26	17140.86	54832.97	175408.51	561125.00
6	19.19	61.40	196.42	628.33	2010.00	6429.91	20569.04	65799.56	210490.21	673350.00
7	22.39	71.63	229.15	733.05	2345.00	7501.56	23997.21	76766.15	245571.91	785575.00
8	25.59	81.87	261.89	837.77	2680.00	8573.22	27425.38	87732.74	280653.62	897800.00
9	28.79	92.10	294.62	942.49	3015.00	9644.87	30853.55	98699.34	315735.32	1010025.00
10	31.99	102.33	327.36	1047.22	3350.00	10716.52	34281.73	109665.93	350817.02	1122250.00

TABLE 9. $b = 396$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	3.31	10.94	36.19	119.72	396.00	1309.89	4332.84	14332.18	47407.97	156816.00
2	6.62	21.88	72.38	239.43	792.00	2619.78	8665.67	28664.36	94815.95	313632.00
3	9.92	32.82	108.58	359.15	1188.00	3929.66	12998.51	42996.55	142223.92	470448.00
4	13.23	43.77	144.77	478.87	1584.00	5239.55	17331.35	57328.73	189631.90	627264.00
5	16.54	54.71	180.96	598.59	1980.00	6549.44	21664.18	71660.91	237039.87	784080.00
6	19.85	65.65	217.15	718.30	2376.00	7859.33	25997.02	85993.09	284447.84	940896.00
7	23.15	76.59	253.35	838.02	2772.00	9169.22	30329.85	100325.27	331855.82	1097712.00
8	26.46	87.53	289.54	957.74	3168.00	10479.11	34662.69	114657.46	379263.79	1254528.00
9	29.77	98.47	325.73	1077.45	3564.00	11788.99	38995.53	128989.64	426671.77	1411344.00
10	33.08	109.42	361.92	1197.17	3960.00	13098.88	43328.36	143321.82	474079.74	1568160.00

TABLE 10. $b = 1507$.

c \ a	1	2	3	4	5	6	7	8	9	10
1	4.32	18.67	80.70	348.73	1507.00	6512.32	28142.24	121613.36	525537.26	2271049.00
2	8.64	37.35	161.40	697.46	3014.00	13024.65	56284.47	243226.72	1051075.52	4542098.00
3	12.96	56.02	242.10	1046.19	4521.00	19536.97	84426.71	364840.08	1576613.28	6813147.00
4	17.29	74.70	322.80	1394.92	6028.00	26049.29	112568.94	486453.44	2102151.04	9084196.00
5	21.61	93.37	403.49	1743.66	7535.00	32561.62	140711.18	608066.80	2627688.80	11355245.00
6	25.93	112.05	484.19	2092.39	9042.00	39073.94	168853.41	729680.16	3153226.56	13626294.00
7	30.25	130.72	564.89	2441.12	10549.00	45586.26	196995.65	851293.52	3678764.32	15897343.00
8	34.57	149.39	645.59	2789.85	12056.00	52098.59	225137.88	972906.88	4204302.08	18168392.00
9	38.89	168.07	726.29	3138.58	13563.00	58610.91	253280.12	1094520.24	4729839.84	20439441.00
10	43.21	186.74	806.99	3487.31	15070.00	65123.23	281422.35	1216133.60	5255377.60	22710490.00

It is now desirable to illustrate the application of this formula by going through the steps of calculating the pathometric index from a particular protocol. The following may be taken as a typical illustrative case.

Autopsy No. 5030. White female. *aet.* 33.

Anatomical diagnosis.

Primary: Syphilitic mesaortitis; aneurysm of ascending arch of aorta; erosion of second and third ribs, right; compression of pulmonary veins; hydrothorax, right; infarcts of kidney and heart; *rupture of aneurysm into pericardium.*

Subsidiary: Fibrous pleurisy; encapulated caseous and calcified nodules in the spleen and left lung.

We then set up the following system of weights:

Organ	Lesion Weight *A*	Organ Weight *B*	Etiological Weight *C*	(From Tables)
Aorta	10	210	8	352800.00
Ribs	6	13	8	173.71
Lungs and pleura	5	396	8	3168.00
Heart	6	210	8	4895.00
Kidneys	3	107	8	132.04
Spleen	2	210	8	67.92
			$\Sigma =$	361236.67
			$\Sigma \div 1000 =$	361.24

Studying the protocol carefully leads to the assignment of the lesion weights in column A. The organ weights are from the table given above. The etiological factor behind all these lesions is infection, either syphilitic or tuberculous.

From Grover's (1921) life tables we find on p. 110 that for white females living in cities in the Registration Area, aged 33-34

$$\mathring{e}_x = 33.20$$

Finally, multiplying 361.24 by 33.20, we get

$$I_p = 11993.$$

It will be seen, in the first place, that with the aid of the tables given above the arithmetical work is reduced to a minimum. By examining the detailed figures it appears that quantitatively the aneurysm accounts for the major portion of the final value of the index, as it ought to, and the contributions of the other lesions to the final result are in about the proportions that common sense would assign them. We have reached a quantitative evaluation of the pathological picture presented by this autopsy.

The Distribution of the Pathometric Index

In order to give some indication of the range of values which this index number I_p may take in actual practice, its value was worked out for 200 consecutive autopsies beginning with autopsy No. 5000. In this series each successive protocol was taken as it came, except that in general all autopsies of still-born infants, and those dying within a few days of birth were omitted, in order that so short a series as 200 might not be unduly weighted with cases of this sort. Also, of course, all incomplete autopsies (mostly brain only) were omitted.

The values of the index for these 200 cases as shown in Table 11, which is arranged in the form of a correlation table with duration of present illness as the second variable. Duration of present illness was extracted from the case histories as the second variable, as an example of general interest in connection with the underlying idea of pathometric index numbers. Is there a significant correlation, when quantitatively measured, between the length of time a person has been ill (*i. e.*, has had clinical symptoms recognizable by himself as existing) and the amount of pathological disturbance found at autopsy?

In this group of 200 cases the index takes values all the way from nearly zero to over 100,000. Over one-half the cases however fall below 5000 in the value of the index. The distribution is plainly extremely skew in the positive direction. A matter of great interest for future work will be to determine the amount and character of variation in the index for groups homogeneous in respect of age. A considerable part of the skewness of the index distribution here shown is undoubtedly due to the fact that in 62 of the 200 autopsies the individual was under 5 years of age.

The biometric constants (which have little meaning because of the deliberately non-homogeneous character of the material in respect of age, race, sex, etc.) from Table 11 are exhibited in Table 12.

TABLE 11.

Values of the pathometric index I_p for 200 autopsies.

	0-4999	5000-9999	10000-14999	15000-19999	20000-24999	25000-29999	30000-34999	35000-39999	40000-44999	45000-49999	50000-54999	55000-59999	60000-64999	65000-69999	70000-74999	75000-79999	80000-84999	85000-89999	90000-94999	95000-99999	100000-104999	105000-109999	Total
Under 1 wk.	15	3	—	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21
1-3.9 wks.	27	8	8	4	5	—	2	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	57
1-5.9 mos.	21	9	9	3	2	3	1	1	1	—	—	—	—	—	—	—	—	—	—	1	—	—	51
6-11.9 mos.	12	3	1	1	—	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	22
1-1.9 yrs.	6	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
2-2.9 yrs.	7	—	2	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	11
3-3.9 yrs.	8	1	4	—	1	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	17
4-4.9 yrs.	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
5-5.9 yrs.	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
6-6.9 yrs.	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
7-7.9 yrs.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	104	27	27	11	8	7	5	1	3	3	—	—	—	—	—	—	—	—	—	1	2	1	200

TABLE 12.

Biometric constants from Table 11.

Variable	Mean	Standard deviation	Coefficient of variation
Pathometric index..	11,225 ± 790	16,560 ± 558	148 ± 12
Dur. present illness	355.2 ± 26.5 d.	555.7 ± 18.7 d.	156 ± 13

Coefficient of correlation = —.071 ± .047.

The constants of variation have high values, presumably in large part due to the non-homogeneity of the material. The correlation between pathometric index and duration of illness is sensibly zero, for this particular sample of material. It may well be that with more homogeneous material a definite correlation would emerge. This will be tested in future work.

Summary

In this paper is presented a preliminary account of a pathometric index number, which has for its purpose the obtaining of a single numerical expression of the gravity of the whole pathological picture disclosed by the lesions discovered at autopsy. The method of calculation is illustrated and the distribution of values of the index for 200 autopsies shown. The purpose of the present paper is purely methodological. Later it is hoped to apply the method to the solution of specific problems.

Literature Cited

1. Glover, J. W. 1921. United States Life Tables 1890, 1901, 1910, and 1901-1910. Washington, Bureau of the Census, pp. 1-496.

2. Pearl, R. 1912. Further notes regarding selection index numbers. Amer. Nat. Vol. 46, pp. 302-307.

3. Pearl, R. 1920. Certain evolutionary aspects of human mortality. *Ibid.*, Vol. 54, pp. 5-44.

4. Pearl, R. and Surface, F. M. 1909. Selection index numbers and their use in breeding. *Ibid.*, Vol. 43, pp. 385-400.

VASO-MOTOR REACTIONS *

By W. M. Bayliss, M. A., D. Sc., F. R. S.

I propose to devote this lecture to a brief consideration of certain recent work, partly my own, on some aspects of the regulation of the circulation. In the first place, the general nature of vascular reflexes requires reference.

To obtain a clear conception of the phenomena, certain general properties of smooth muscle and its innervation must be kept in mind. Like this tissue in other places, the arterial muscle tends naturally when left to itself to be in a state of moderate contraction or "tone." There are various phenomena which demonstrate this fact, but perhaps the best known is that the blood vessels of an organ, after the nerves are cut, can be made either to relax or contract by stimulation of the peripheral ends of certain nerves supplying the organ. Thus, the arterial muscle is supplied with excitatory and with inhibitory nerves. We call the former, vaso-constrictors, the latter, vaso-dilators. It appears that the former always come from the sympathetic system; the latter from various other sources, but leaving the nervous system in cerebral and spinal nerves. There is usually a certain degree of constant activity of the vaso-constrictors, by impulses from the nerve-centres, probably of reflex origin. The vaso-dilator nerves are only in tonic action under special conditions, those of the skin, for example, under hot and moist states of the atmosphere. The latter appear also to be active in the depressor reflex excited by high blood pressure or an over-full state of the vascular system. This reflex fall of blood pressure brought about by stimulation of the central end of the depressor nerve is familiar

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to all and we may take it as the vascular reflex most completely worked out.

In theory, a fall of blood pressure due to vaso-dilatation, can be obtained in either of two ways—by inhibition of the vaso-constrictor centre when it is in activity, or by excitation of the vaso-dilator centre. Ludwig and Cyon, the discoverer of the depressor nerve, ascribed the effect to the former; Heidenhain and Ostroumov to the latter. The difference of opinion may perhaps be due to the former observers having directed their attention chiefly to the visceral circulation, the innervation of which is preponderantly vaso-constrictor, while the latter observed chiefly the skin, whose innervation is mainly vaso-dilator. In some experiments which I made several years ago, the impression was given me that both components mentioned played a part throughout. This suggestion was made before the famous work of Sherrington on reciprocal innervation in reflexes to voluntary muscles. Since my earlier experiments had shown the probability of a kind of reciprocal innervation in vascular reflexes, I took up the question again, especially as Sherrington had found that strychnine reversed the inhibitory effect of the depressor nerve, as it did the inhibitory component of reflexes to voluntary muscles, and had suggested that I should work it out further.

Taking first the depressor reflex, I found that not only was there an inhibition of the constrictor centre, but also an excitation of the dilator centre. The first was shown by vaso-dilatation in regions from which the vaso-dilator innervation had been cut off, as the hind leg after removal of the lumbar cord; the second, by the presence of dilatation in regions from which the constrictor innervation had been removed, as the submaxillary gland after section of the sympathetic supply. Conversely, in the pressor reflexes from ordinary sensory nerves, there is not only an excitation of vaso-constrictors, but also an inhibition of vaso-dilators. This last fact is the most difficult to demonstrate, because it requires the presence of tone in the dilator centre, which is not the usual state of affairs. A similar difficulty was met with by Sherrington in certain of his experiments.

The effect of strychnine was found to consist in a conversion of the inhibitory component of both kinds of reflex into an excitation, while that of chloroform is of the opposite kind, namely, a conversion of excitation into inhibition. These effects of the two drugs were found to explain all the puzzling results met with after evisceration, and other interferences. In the eviscerated animal, with all the accessible vaso-constrictor nerves also cut, we have a preparation in which all the vascular reflexes are effected by the vaso-dilator centre. Since in the depressor reflex, there is excitation of this centre, it is easy to see that strychnine cannot reverse the depressor in these circumstances. Pressor reflexes, in which there is normally an inhibition of the dilator centre, become, after strychnine in the "dilator" preparation, depressor in nature, owing to conversion to excitation of the dilator centre.

I venture to think that E. K. Martin in a recent paper has misunderstood the evidence for reciprocal innervation. It is not merely a question of balancing a pressor against a depressor response, but of the fact that in say a depressor reflex, produced in the same way in both cases, it can be shown that there is brought about both excitation of vaso-dilator nerves and inhibition of vaso-constrictor tone. The relative importance of the two in any particular organ depends on the preponderance of the one or the other kind of innervation.

Before leaving the subject of vascular reflexes, I would call attention to a not-infrequent source of error in the interpretation of peripheral effects. This is the failure to realize that a region which is larger than another or more copiously supplied with vaso-motor nerves, may affect this other by draining away or supplying more blood; so that, for example, if there is simultaneous reflex constriction in the viscera and in the skin, it may appear as if there were dilatation, or absence of effect in the skin, whereas repetition of the experiment in the eviscerated animal, or with an arrangement to prevent rise or fall of general blood pressure, shows that there was really constriction in both. Unless this test is applied, no conclusion as to opposite effects in the splanchnic and limb circulations can be made, although it is often done.

The peculiar nature of the vaso-dilator innervation of certain regions of the body may next attract our attention. Stricker in 1876 stated that when the peripheral ends of the posterior roots of the sacral plexus in the dog were stimulated, the temperature of the paws rose owing to vascular dilatation. Although this result was confirmed by some later workers, it was not generally accepted, probably because the methods adopted did not seem sufficiently free from objection to warrant acceptance of a contradiction of the Bell-Magendie law. At a still later date, when investigating the course of the vaso-dilator fibres of the hind leg by the plethysmographic method, I took occasion to test the effect of stimulating the dorsal roots and found to my surprise that Stricker's statement was quite correct, even when the spinal cord was cut out and mechanical stimuli used, so that all possible error seemed to be obviated. I was inclined at first to attribute the result to aberrant spinal efferent fibres, until I found that the effect was not abolished by section of the posterior roots between the ganglion and the cord and subsequent degeneration of any possible spinal efferent fibres. They did degenerate, however, when the ganglia were removed. Thus, the fibres in question were anatomically identical with the ordinary sensory fibres. At Prof. Langley's suggestion, the effect was called "antidromic," because of its being due to impulses passing along the sensory fibres in a direction opposite to the usually accepted one. The nature of the peripheral connections seemed puzzling and I suggested the possibility

of a peripheral plexus from which fibres passed on the one hand to arterioles, on the other to sense receptors, but thought it improbable on account of the reaction time being expected to be short on such a view. However, subsequently, Ninian Bruce showed that the process had the characteristics of an "axon-reflex" in the sense of Langley and Anderson, as it would be on the hypothesis mentioned, although a branched fibre would suffice, without the peripheral plexus. The facts brought out in Bruce's work are of interest in themselves. Spiess had shown that the irritant effect of oil of mustard on the skin is absent if cocaine has been applied. Since cocaine paralyzes sensory endings, it was thought that a spinal reflex of a vaso-dilator nature was involved. Bruce found on the contrary that the irritant effect was still present and paralyzed by cocaine if the trunk of the nerve to the region was cut. It was therefore independent of the spinal cord, not a reflex. It was abolished, however, if the nerve was allowed to degenerate. No other explanation than that of an axon-reflex is possible. Impulses pass from a sensory receptor up the nerve as far as the point where a branch is given off to an arteriole and then pass both onwards to the cord and backward to the blood vessels. Innervation from the cord passes both to the arterioles and to the sensory organ, but presumably does not reach beyond some synapse in the latter place.

This antidromic axon-reflex effect has recently become of interest in connection with the innervation of the capillaries. There was at one time felt to be a difficulty in supposing independent contractility of these vessels on account of their protoplasmic nature. But this is not serious, since cells like those of the amœba are able to change their shape, and this is all that is needed. Roy and Graham Brown saw neighboring capillaries change their diameter independently of one another, so that the effect could not be one of mere passive response to arterial pressure. Krogh has recently investigated the matter in more detail. He found that stimulation by touching with a pointed needle caused a local dilatation, which spread along the capillary to a little distance. After the application of cocaine or degeneration of the nerve supply, the effect was limited to the point stimulated. The similarity to the antidromic phenomenon described previously is obvious and Krogh interprets it in that way. Starling has suggested that the whole of the antidromic effect might be situated in the capillaries. Under his direction, Doi made some experiments on the question. He found that the dorsal roots of the frog have an antidromic dilator effect; earlier observers had overlooked the fact on account of the very slow time course. Doi showed that it was still present after maximal dilatation of the arterioles by acetylcholine or of the capillaries by histamine. Hence it appears to be common to both.

The action of histamine, as investigated by Dale and Richards, has thrown much light on the properties of the capillaries. Dr. Dale has given an account of this work in a previous Herter lecture, so that I may content myself with reference to its property of dilating capillaries, while causing contraction of plain muscle, including that of the arterioles. An important additional effect of histamine in large doses is to increase the permeability of the capillaries. This has its significance in respect of the symptoms present in severe wound-shock, as we shall see later.

Antidromic phenomena have a clinical interest in respect to Herpes. The work of Head and Campbell located the lesion in the dorsal root ganglia. The problem arises as to how far mere vascular dilatation can produce destruction of tissue, such as blisters and so on. Does persistent irritation of a nerve cause violent peripheral effects? This seems to suggest the need of experimental work.

Some recent work by Burn on pilocarpine has emphasized the importance of the capillary circulation for normal peripheral effects. The failure of the sweat glands to respond to this drug in certain nervous conditions has been a puzzle. Burn found that section and degeneration of the sympathetic supply to the cat's paw did not affect the response, whereas that of the main sciatic nerve usually abolished it. The reaction of the capillaries to histamine was also absent whenever that to pilocarpine was absent. In the course of regeneration, the reaction to pilocarpine returns at an early date, corresponding to that found by Head for the protopathic sensory fibres and much earlier than that of the sympathetic, motor, or epicritic fibres. In some experiments made by Head and myself, we found that the antidromic vascular dilatation from the peripheral end of the regenerating radial nerve was present at a time when only protopathic fibres had made their appearance. The facts as a whole suggest that the antidromic innervation of the peripheral circulation is necessary to the normal activity, not only of the capillaries themselves, but indirectly of the tissues in general.

I wish next to call attention to an aspect of the circulation which is apt to be unduly neglected, namely, the results which follow from changes in the volume of the blood in relation to the capacity of the vascular system which contains it.

Let us recall the fact that the amount of blood propelled by the heart depends on that reaching it by the veins, or in other words, on how much the ventricles contain at the moment of systole. It is clear that with a given total volume of blood, the more is contained in peripheral regions, the less must be in the heart. Hence, the amount in effective circulation is diminished and the arterial pressure is low. This aspect of capacity of the vascular system was always insisted upon by the Ludwig school and work done by Starling and myself confirmed their views. Mere consideration of rate of flow through dilated or constricted arterioles may lead to mistaken conclusions. For example, depressor stimulation causes an increase in this rate of flow and it might be expected to

cause a rise in the pressure in the vena cava, along with increased inflow into the heart and so on. This is not the case, however, except for a brief period; the increased capacity of the arterial system more than compensates for the increased rate of flow. Blood is held back in the enlarged arterial space.

This aspect of the circulation has assumed practical importance in relation to wound-shock, owing mainly to the work of Dale and Richards on the capillaries. When we remember the relatively enormous proportion of the capillary part of the vascular system to the whole, we realize how a comparatively small dilatation of these vessels, if widespread, may lead to a very serious drainage of blood into them, with loss of blood from effective circulation and all the consequences of deficient supply to vital organs. Practically all the symptoms of wound-shock can be accounted for on this basis.

Observations by means of the vital red method on men wounded in the war showed a deficiency of blood in circulation. This was due partly to capillary dilatation; partly, in later severe stages, to loss of plasma owing to the capillaries becoming permeable not only to salts but also to colloids. The general effect is similar to that of hæmorrhage and it was often impossible to distinguish between them.

So long as the increased permeability had not progressed too far, treatment of hæmorrhage and shock without hæmorrhage by transfusion of blood or by 6 per cent solution of gum acacia in 0.9 per cent sodium chloride was found effective.

The necessity of a colloid with an osmotic pressure equal to that of the blood proteins will be obvious from what was said in my first lecture. Salt solutions rapidly pass out from the blood vessels. The osmotic pressure of the colloids in blood or in gum-saline causes the fluid injected to be retained. [Tracings were shown of the different results of the two kinds of solution.] It is plain that unlimited loss of blood cannot be replaced by any solution devoid of hæmoglobin. The limit was found experimentally to be about when half of the blood had been removed. Such cases practically do not occur in practice and the experiments of Haggard and Henderson lately published do not bear upon the problem of the practical use of gum-saline, since in their experiments the hæmorrhage was in excess of that claimed to be capable of replacement. It is only in cases of excessive loss of blood that the factor of importance is the hæmoglobin. The real factor in other cases is the volume of the blood.

Experimental work on cats showed that gum-saline was effective in the state of shock produced by massive injury to muscular tissue. This was also found to be the case in many instances of wounds in the war. But there was a general impression that after the state of increased permeability had developed, treatment was hopeless. It was found that even blood left the circulation rapidly and was useless. But, at the same time, certain experiments which I made on cats in histamine shock gave me the impression that if repeated injections of moderate volume at half-hour intervals or so had been tried, more favorable results would have been obtained. It appears that the first injections, although more or less rapidly lost, set the blood vessels by improved circulation through them on the way to recovery. Incidentally, gum-saline has an advantage here, since it is scarcely possible to arrange for repeated transfusions of blood.

Naturally, the hæmoglobin left in the circulation is diluted by an artificial fluid and it is perhaps surprising that the result is as good as it is. The explanation lies no doubt in the fact brought out by Gesell, namely, that the actual amount of hæmoglobin, etc., carried through a tissue in a given time is really increased. The greater rate of flow, owing to the larger volume in circulation more than compensates for the dilution. The "nutrient flow," as Gesell calls it, is increased.

After careful consideration of all the objections brought against the use of gum-saline, I am convinced that any unfavorable results are due either to non-observance of the methods which I have given for the making of the solution, or to its use in cases in which nothing whatever would have served. Of course, it cannot be expected to replace unlimited loss of blood. But, as much as two-thirds of that in the body can be effectively replaced. This implies a loss of so great a magnitude as rarely happens.

It seems probable that determinations of the blood volume will be found to give valuable clinical information. It is not necessary to use vital-red; the observations of D. T. Harris and others have shown that the easily accessible congo-red is really superior, since it leaves the blood vessels less rapidly. Owing to its cheapness, it can be purified, since the considerable incidental loss is immaterial.

The last matter to which I would refer, is some recent work on functional vascular dilatation. The increased supply of blood required by an active organ may be afforded in two ways—either by stimulation of vaso-dilator nerves from the centre, or by some chemical products formed by the activity of the cells. Since it is certain that the latter takes place, it has been thought by some that there is no need for the assumption of vaso-dilator nerves. I showed that carbonic acid, a universal product of cell activity, causes dilatation of the blood vessels. Gaskell had already shown that lactic acid acts in the same way, and lactic acid has recently been shown to be present in the venous blood from the tongue when its muscle contracts in response to stimulation of the hypoglossal nerve. The effect seems to be a general property of acids, that is of hydrogen-ion. From certain remarks made by Barcroft, a nitrogenous product of the nature of histamine appears to be in his mind. As to this, we have no evidence of a nitrogenous product in the normal activity of muscle. Moreover, capillary dilatation would be ineffective without arterial dilatation. In fact, acetyl-

choline would be appropriate. But we have as yet no evidence that it plays a part.

In this connection, two kinds of action of external cold on the skin are to the point. One of these, which may be regarded as the healthy reaction, is a red warm skin. There is arterial dilatation, which sends a rapid current of warm oxygenated blood through the capillaries and protects the skin from frost-bite. In the other, abnormal, reaction, the capillaries are also filled with blood, but the skin is blue and cold. Although the capillaries are dilated, the arterioles are contracted, so that the current of blood is slow, it becomes cold and loses its oxygen, becoming venous and blue. Capillary dilatation alone is thus insufficient to ensure a good circulation. I am inclined to think that the fall of blood pressure caused by histamine is due chiefly to a capacity effect of draining blood into the capillary region, and only to a small degree to a lowered peripheral resistance. A slight increase in the wide bed of the capillaries would have but little effect on the resistance, in comparison with that produced by changes in the arterioles.

There is also evidence that lactic acid is formed in the asphyxial condition of cells. If this compound is a stage in the oxidation of glucose, it is quite likely to remain as such in the absence of sufficient oxygen to burn it completely. The vaso-dilatation produced by this acid has its interest in relation to the response of arterial muscle to changes of internal pressure. My original experiments were made before the discovery of the reflex liberation of adrenaline by stimulation of sensory nerves, and were inadequate to prove the reaction to stretching by rise of pressure. The dilator response to lowered pressure may have been due to asphyxial products, but in view of the conditions of the experiments, I think that this explanation is not altogether satisfactory. Further experiments on isolated arteries are needed, but my experience has been that it is a matter of difficulty to obtain these in a sufficiently responsive state. They readily pass into a contracted state, in which they seem inexcitable.

The actual existence of vaso-dilator nerves apart from "metabolites" has been demonstrated by some recent experiments by Anrep and Evans and by D. T. Harris. It was found that the lingual nerve produced a large dilatation in the tongue without any increase in oxygen consumption; that is, without any increase in cell activity. In fact, in Harris's experiments, brief stimulation of the lingual caused a much greater dilatation than a corresponding one of the hypoglossal, whose effect is only caused by products of activity of the muscle. The dilator nerves of the dog's tongue come into play especially in temperature regulation.

It is an interesting question whether innervation of dilator nerves is a part of the coordinated reflex to various effector organs, gland or muscle. Anrep found that the reflex dilatation of the submaxillary gland, brought about by application of acid to the tongue, was as great after atropine as before. Hence, the vaso-dilator mechanism was set in action reflexly, apart from the production of "metabolites."

NOTES ON NEW BOOKS

A Textbook of Gynæcological Surgery. By BERKELEY and BONNEY. 829 pp. 2nd edition. Cloth, $11.00 (*N. Y., Paul B. Hoeber, 1920.*)

It is a pleasure to turn over the pages of a book that is as well prepared as this one. The subject matter is for the most part presented in an up-to-date and clean-cut manner. The illustrations, although not elaborate, on the whole are clear and supplement the text well. To a large extent one of the commonest faults of recent gynecological text-books has been avoided—the tendency to distort the sense of balance and proportion by laying undue stress on minor pet procedures in which the authors are particularly interested. The sections on post-operative complications and care and on ultimate results add materially to the value of the work.

As the authors state, this book presents the opinions and practices of the school of gynecology to which they belong. In general, we feel that these views are sound and trustworthy, although quite naturally ours differ from theirs in some particulars. Thus, in our opinion they have underestimated the advantages that may at times be gained by draining the pelvis through the cul-de-sac of Douglas rather than through the abdomen, and they have also failed to appreciate the value of posterior colpotomy as a diagnostic or therapeutic procedure in selected cases. We would criticize their treatment of perineal plastic surgery as being incomplete. It seems unusual that more space should be devoted to the directions for dilating the cervix (fourteen pages) than to the treatment of vesico-vaginal fistula (twelve pages). Little or no attention is paid to the subvesical fascia in the treatment of cystocele, vesico-vaginal fistula or prolapsus; this may be the cause of some of the failures that they seem to have had. The treatment of complete laceration of the perineum is too cursory to be sufficient; not enough attention is paid to the rectal sphincter or the obtaining of continence, which is usually the only indication for performing the operation. In the treatment of carcinoma of cervix, the authors are far more radical than are most gynecologists of the present day; it is an open question whether the ultra-radical operation involving the wide dissection of the pelvic and iliac glands, the parametrium and vagina is worth while. Their method of taking care of the vagina both before and after perineal operations is rather elaborate; in our experience perineal infections have been much less common since we have simplified our technique. We would question the wisdom of devoting space to operative procedures that are definitely out of date, such as Le Fort's operation for uterine prolapse or the making of a common cloaca out of the vagina and bladder in some cases of vesico-vaginal fistula. Their indications for performing hysterotomy and utriculoplasty also seem questionable. In their treatment of carcinoma of the rectum, they recommend that the combined abdominal and perineal operation be performed in one stage; in some cases, at any rate, it is undoubtedly safer to carry this out in two stages.

From what we have said, we would not wish the reader to get the idea that this is not a good book. On the contrary, it strikes us as being one of the best recent text-books on operative gynecology. With the above reservations, we can recommend it both to the expert gynecologist and to the beginner, for both will find in its pages much that is interesting and instructive.

L. R. W.

The Diseases of Children. By SIR JAMES F. GOODHART and GEORGE FREDERICK STILL. 11th Edition. Cloth, $10. (*New York, Paul B. Hoeber, 1921.*)

This is the sort of book which is and was meant to be a text-book of pediatrics for students of medicine and should be judged as such. How well it has served its purpose in the past is indicated by the fact that this is the eleventh edition since it first made its appearance in 1885. In this last revision of the book Dr. Still has studied to retain as much of the text left by Sir James Goodhart as possible and still to bring it abreast of the present day.

The general consideration of the diet and hygiene of the healthy child is very well done and the author offers a reasonably simple, if somewhat dogmatic, system for the modification of milk for the hand-fed infant. The book is untouched by post-war fads which have grown up out of plausible empiricism masquerading as science. Their omission may stamp the book as obsolete to the beginner in whose novitiate they had their rise and before whose heyday they will pass into the limbo of the forgotten. After all, the question which matters in feeding a baby is not what *may* be given but what is the *safest* food for the average child. To teach this is all that can be attempted in a text-book. Experience only can teach a student the art of dealing with the idiosyncrasy of the individual child so as to secure optimal results. Still's feeding is based on the percentage composition of milk, and his formulæ are made up with added cream. An infant fed according to this book would receive a good bit more fat than is usually allowed by American pediatrists nowadays and the interval between the feedings is shorter than that which has found favor in this country. Nevertheless, the book offers the student a tolerable ground on which to base further study.

The pathology, symptomatology and diagnosis of the morbid conditions of childhood have been thoroughly and carefully set forth. Considerable less emphasis has been placed by the author on laboratory diagnosis than is found in most American books on medicine. As regards etiology, the book is not equally good throughout. For example, the author has gone into the etiology of poliomyelitis with some care and has reported it at sufficient length. On the other hand, if the text may be used as an index, he has entirely ignored the recent work which has been done to reveal the factors which underlie the occurrence of tetany. The chapters on the exanthemata are excellent.

In the treatment of the disease the author is much more prone to rely on administration of drugs than is customary in this country, possibly because he knows them better. There can be no doubt that in the hands of a competent therapeutist the simples and compounds of the pharmacist contribute much to the safe and happy curing of disease, and the list of prescripts at the end of the book contains many excellent formulæ. Drugging is, however, easily overdone and many of the diseases to which children are subject may be confidently and safely handled by regulating the diet and hygiene of the patients. It is well to remember that if one is to have recourse at all to the pharmacopeia in the practice of pediatrics, one should be qualified by a much greater knowledge of pharmacy, pharmacology and therapeutics than the average physician possesses.

Diluis helleborum, certo compescere puncto
Nescius examen—

In closing, a protest is in order against the exorbitant prices which are charged in America for English books. Ten dollars is far too high a price for this text which, although it is printed well and on good paper, is not expensively illustrated and is wretchedly bound with the cheapest possible material. In such a dress the book would be dear at half the price now charged for it. Not only should books be cheaper but they should be better made. By far the greater number of those published now will be dust before fifty years are past and the book which is worth publishing at all is worthy of publication with a view to a longer life. It may be that the useful existence of a modern book of medicine is but seven years. Granted that this is so, such a statement only supplies an argument for the preservation against time of the only available records of the period of change and investigation which is responsible for its truth.

P. G. S.

Modern Surgery, General and Operative. By JOHN CHALMERS DACOSTA. Cloth, $8.00. (*Philadelphia and London, W. B. Saunders Co., 1919.*)

The appearance of a new edition of DaCosta is always most welcome. Little comment is necessary or desirable on this wonderfully concise, accurate and up-to-date volume. For a subject so extensive, it is always a marvel how it can be done so well.

W. E. D.

Submucous Resection of the Nasal Septum. By WILLIAM MEDDAUGH DUNNING, M. D. (*New York, Surgery Publishing Company, 1922.*)

This book contains forty-seven pages of text and twenty-five illustrations. The author discusses briefly the anatomy and physiology of the nose, and considers, in a somewhat cursory manner, the etiology of septal deviation and its relation to such general disorders as hay fever and asthma. He classifies septal deviations as (1) bowed or curved, and (2) angular, and describes the operative procedures he has devised for their correction. The schematic drawings illustrating the various steps of the operation could be improved upon. At the end he presents a few case histories which show the beneficial effects that result when the operation is really indicated and is properly performed.

The facts could have been presented in a much more concise manner, and while there are many interesting and helpful suggestions, there is really little that has been added to what we find in the standard text-books on rhinology.

J. W. B.

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